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EXTRACTION AND STANDARDIZATION OF PITUITARY FOLLICLE-STIMULATING AND LUTEINIZING HORMONES[1]

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WE HAVE PREVIOUSLY reported general methods for the extraction of the gonadotropic hormones from acetone desiccated pituitary tissue and the fractionation of the extract into two gonadotropic fractions (1, 2, 3, 4). One of these preparations (FSH) produces follicular growth in the ovaries and tubular development in the testes while the second (LH) produces corpora lutea if injected with the FSH or if given to animals whose ovaries have undergone considerable follicular development. This preparation does not produce follicles or corpora lutea when injected alone into sexually immature, 21-day-old, or hypophysectomized rats. In the male, this preparation acts on the interstitial elements of the testes causing secretion of male hormone, resulting in hypertrophy of the secondary sex glands. Because of the selective action of these two preparations on the male and female gonads, we concluded that each preparation contained a separate gonadotropic hormone and that the gonadotropic activity of the pituitary is carried out by these two factors. This conclusion has been confirmed by Wallen-Lawrence (5) and also by Evans et al. (6,7), who, however, report that interstitital stimulation is a property of a separate factor from that which produces luteinization.

It is the purpose of this communication to describe in some detail the methods used in the extraction and purification of the two hormones and also to point out some of the methods which we have developed for the characterization and assay of gonadotropic extracts. The extraction procedure which is described in this paper has been worked out chiefly for fresh sheep pituitaries but applies equally well to desiccated tissue except that the volume of extractive in proportion to tissue must be increased 5-fold.

The extraction of the gonadotropic hormones from pituitary tissue is in

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general best accomplished by alkaline aqueous extractives but very good yield of both factors is obtained even at pH 6.0 (4). A number of alkalies have been used such as saturated barium hydroxide (6) 0.02N ammonium hydroxide (5) and 50% pyridine (1,2,3). The hormones are decreasingly stable with increasing pH above pH 9.0 consequently it is advisable to use weak alkalies. We have previously used 50% aqueous pyridine but have now found that a weaker solution (2%), while a little slower in action offers several advantages. The use of pyridine, in addition to its alkaline properties, offers the advantage of being an effective preserving agent and while extraction of fresh tissue is invariably carried out in the cold, putrefaction is prevented even at room temperature for two days.

To determine whether any destruction of activity took place due to the solvent, standardized pituitary powders were treated for 2 days with 5-gram volumes of 2% aqueous pyridine. Six volumes of cold acetone were then added and the insoluble material collected by centrifuging, powdered and restandardized. The restandardization of the powders after treatment showed that the activity had increased, especially so in the case of the horse pituitary[2] where the increase was at least 300% (table 1). Although at present there is no obvious explanation for this, the probability is that it may be related in some way to the availability of the hormones.

TABLE 1. ACTIVITY OF PITUITARY POWDERS BEFORE AND AFTER TREATMENT WITH 2% AQUEOUS PYRIDINE AND OF THE PYRIDINE EXTRACT OF THE POWDERS

	Amount	Weight of Ovaries		
		Before	After	Extract
	mg.	mg.	mg.	mg.
Horse pit. powder	1	20	87	80
	2	25	95	97
	3	45	123	110
Sheep pit. powder	10	22	31	28
	15	29	43	32
	20	43	56	41
Hog pit. powder	15	22	26	20
	25	33	42	30
	50	67	81	61
Rat pit. powder	5	18	27	28
	10	32	57	39
	20	55	88	63

The efficiency of two per cent pyridine as an extractive was tested by extracting similar standardized pituitary powders two times with 5-gram volumes, removing the insoluble residue by centrifuging after each extraction. The extracts were then combined and assayed (table 1). The extracts of hog and sheep pituitary powder were somewhat less potent than the treated glands, while those of horse and rat were approximately equal in activity to the treated tissue. From the results in table 1 it is difficult to evalu-

[2] We are very grateful to Dr. A. S. Parks for the horse pituitary powder used in these experiments.

ate accurately the efficiency of the extraction since we know that absorption rates and consequent utilization efficiency can be changed greatly by the presence of inert material. In the case of the horse pituitary powder it would appear that the extraction was very nearly 100% complete whereas in the others the yield was somewhat lower.

TABLE 2. ACTIVITY OF PITUITARY POWDERS AND OF 2% PYRIDINE EXTRACTS OF THE POWDERS AS DETERMINED BY OVARIAN WEIGHTS AND BY SEMINAL VESICLE WEIGHTS

	Female Units per kg.	Male Units per kg.	Ratio
Horse pituitary powder	1,000,000	60,000	16:1
Horse pituitary extract	5,000,000	250,000	20:1
Hog pituitary powder	60,000	120,000	1:2
Hog pituitary extract	50,000	150,000	1:3
Sheep pituitary powder	100,000	400,000	1:4
Sheep pituitary extract	80,000	400,000	1:5

Since we are dealing with two gonadotropic factors it was also of interest to determine whether the crude extract contained the two hormones in approximately the same proportions as the powdered glands. This was determined by using the seminal vescicle response as a rough measure of the LH while the relative amount of FSH was determined by the ovarian development in immature female rats (see below for methods of standardization). The results show that according to these tests the proportion between the two remains approximately the same within the limits of accuracy of the assay method so it seems that the solvent removes both hormones with equal efficiency (table 2). The relative proportions of the two hormones differ markedly in horse pituitary powder and in those of hog and sheep.

Method of Extraction

The method for extracting fresh pituitary tissue is outlined in the accompanying diagram. The removal of the extract from the tissue pulp may be accomplished by centrifuging the material without neutralization, but adjusting the solution from pH 7.0 to 5.6 with acetic acid causes only slight loss of activity and makes the separation much sharper and in addition gives an extract much lower in solids.

The concentration of the combined extracts may be accomplished in at least three different ways with equal efficiency so the method used may be determined by the preference of the individual worker. It is rather difficult to concentrate the extract under vacuum because of violent foaming. However, we have made use of a special type of evaporator which permits the evaporation of the solvent with a minimum of trouble. This consists merely of a metal tube, inserted from the side and extending across the back of a chemical hood, open on the outside but closed at the end inside the hood. From the sides of the tube several vents, depending on the size of the hood, extend downward to within 4 or 5 inches of the floor of the hood. The solution to be evaporated is placed under these vents in large evaporating dishes and by closing the hood the air is drawn through the vents over the solution.

The temperature of the evaporation is regulated by a cone heater placed near the open end of the tube outside the hood. By inserting the heater within the tube or by moving it away from the opening, a wide variation of temperature may be obtained. The evaporation of the AP extract proceeds rapidly at a temperature of 37° C. and no loss of activity is apparent. No attention is required during the course of the evaporation except toward the end, when, if the material is allowed to become too dry some will be blown out of the dishes.

A second method of concentration of the crude extract is by precipitation with cold acetone. The hormones are insoluble or practically so in 80% acetone so the extract is poured into 4 volumes of cold acetone with stirring. The material ordinarily precipitates readily but sometimes a colloidal suspension is obtained. This may be flocculated by the addition of a few drops of saturated sodium chloride solution followed by shaking. The material may be collected by centrifugation but filtering with suction is preferred chiefly because the residual acetone is more completely removed from the precipitate, and moreover the filtration is more rapid than centrifugation. All these procedures are carried out with cold acetone in the cold room but we have not been able to detect any destruction of activity through denaturization even when the same precipitation was performed at room temperatures.

The third method which we have used for concentration is the precipitation of the active material by saturation of the solution with ammonium-sulfate. The active principles being protein-like in nature are precipitated and are collected by filtration. The filtration is best accomplished by using a pad of celite in a Buchner funnel, this being approximately three times more rapid than filtration through hardened filter paper. The precipitate forms a cake over the celite which may be removed, leaving practically all the celite behind.

The next step in the purification takes into consideration the fact that the gonadotropic hormones are soluble at a pH of 5.0 whereas much inert material together with the lactogenic and adrenalotropic hormones is insoluble. If the salting out procedure has been used in the concentration of the crude extract, the emulsified precipitate is dialyzed to remove all salt as more precipitate is brought down at pH 5.0 in a salt-free solution. The dialysis is carried out through cellophane tubes, and the solution in the tubes is stirred during the course of the dialysis making use of the apparatus designed by Kunitz (8). The dialyzed solution is then carefully adjusted to pH 5.0, centrifuged and the precipitate washed with distilled water. The residue is practically free of gonadotropic hormones but contains lactogenic and adrenalotropic activity.

In one of our earlier papers (2) we reported that benzoic acid removed part of the luteinizing hormone from a solution of FSH and LH. The LH is adsorbed from solution more readily than the FSH, but it is also possible, after removal of the benzoic acid from the precipitate by washing with acetone, to extract the gonadotropic hormones from this precipitate with distilled water leaving behind a voluminous precipitate that is inactive, as far

as gonadotropic activity is concerned. The combined solutions contain both the FSH and LH.

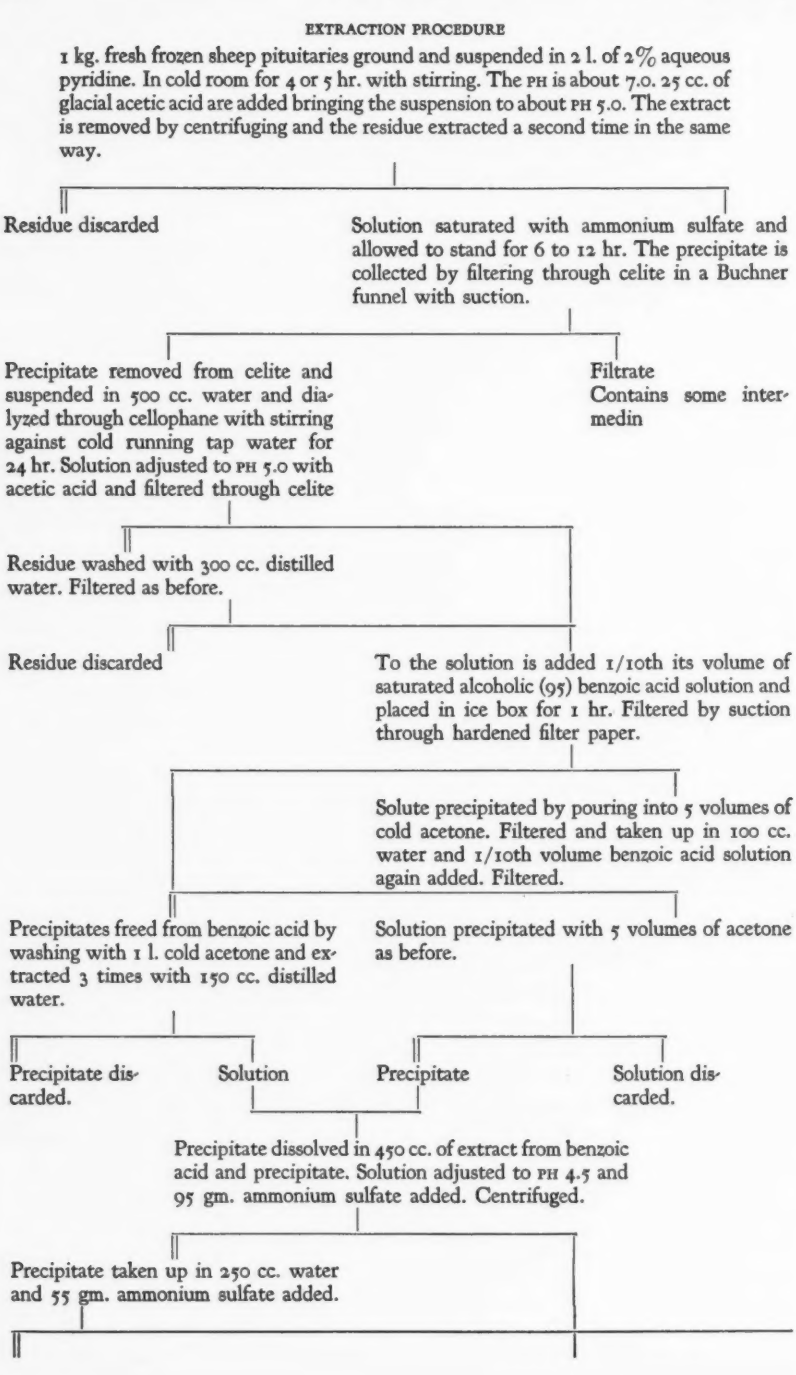
The separation of the two hormones is made possible by their differences in solubility in aqueous solutions. The presence of the two factors was originally observed when part of the LH remained in the insoluble fraction after extraction of a gonadotropic precipitate with distilled water (1). Later we reported that quite a complete separation of the two substances was accomplished at pH 4.2 in the presence of acetone or alcohol or ammonium sulfate or 1% cresol (3,4). A rather disturbing fact was noted when we began using fresh pituitary material instead of the acetone desiccated powder. When the powder was used the isoelectric precipitate at 4.2 was very active for luteinization and could be redissolved and reprecipitated with no apparent loss of activity. Consequently we came to the conclusion that LH was insoluble at pH 4.2. However, when fresh tissue was employed as the source, and particularly after purification from inert insoluble material, the solubility is much greater and even at pH 4.2 the LH does not flocculate readily from pure aqueous solution but does so in the presence of 25 to 40% acetone or if the solution contains 21% ammonium sulfate. At pH 5.0 or above the LH is soluble under these conditions.

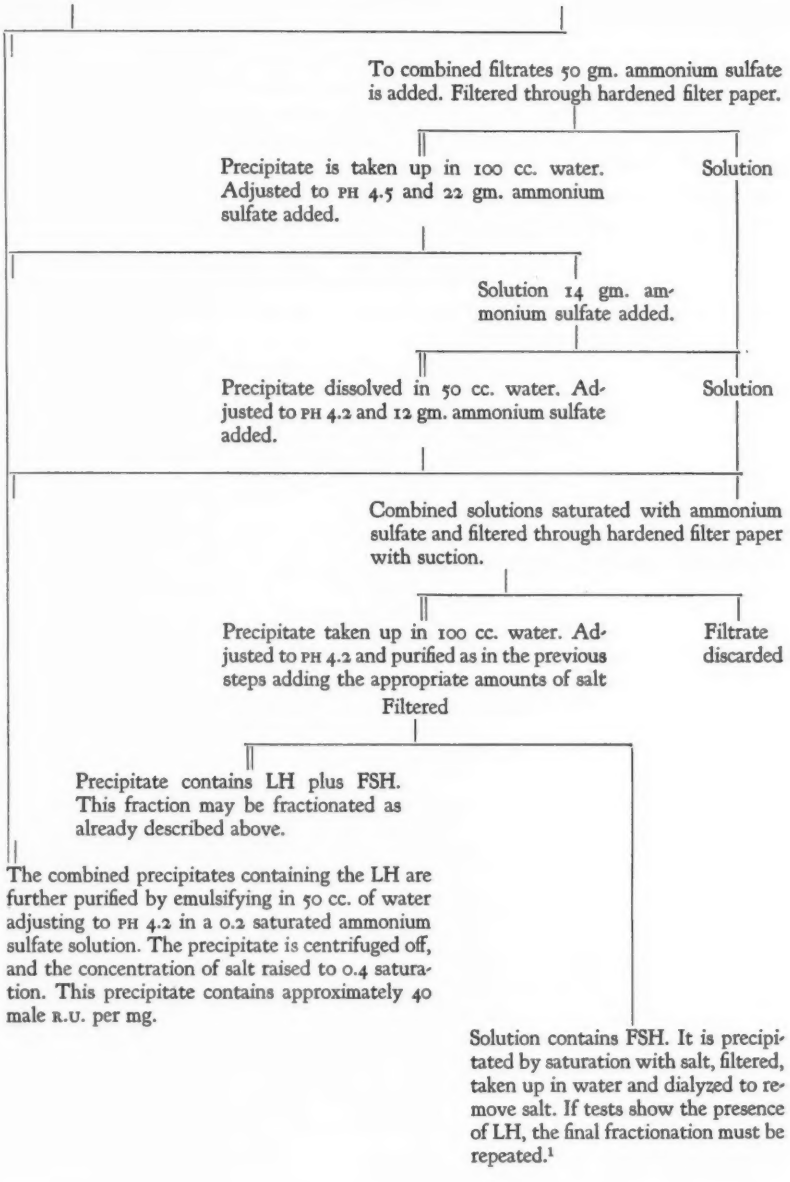
It should be made clear that the separation of the two factors is not sharp. We have never been able to find any pH or any solvent in which one hormone is soluble and the other insoluble to the extent that a sharp separation could be effected. The separation of the two factors depends on fractional precipitation and their solubilities overlap to a considerable extent, so much so that it is only by repeated precipitation and solution that preparations of one gonadotropic hormone are obtained which are free of the other factor. Thus at pH 4.2 in 21% ammonium sulfate solution, a certain amount of the LH precipitates out which is practically free of FSH. If now the amount of ammonium sulfate is increased to 30 or 35% a second precipitate comes down which contains mostly LH but also has appreciable quantities of FSH. The material in solution is primarily FSH but it must be precipitated by saturation with the salt, redissolved and again fractionated before it is free of LH. Similarly the LH fractions must be precipitated several times before all the FSH is removed as determined by the inability of the preparation to promote ovarian development in the immature rat when given alone.

A number of other methods have been employed in the further purification and concentration of the active principles. However, no method has been perfected which permits a rapid and easy separation of the two factors. Differential absorption has proven somewhat effective as has also differential precipitation with protein precipitants. However, the same problem of the overlapping of solubilities is present in these methods and so far at least no specific chemical reagent for separating the two hormones has been found.

In addition to the solubilities another chemical difference between the two substances has recently been found. Picrolonic acid precipitates both substances and the LH is as active as before precipitation. The FSH precipi-

EXTRACTION PROCEDURE


1 kg. fresh frozen sheep pituitaries ground and suspended in 2 l. of 2% aqueous pyridine. In cold room for 4 or 5 hr. with stirring. The PH is about 7.0. 25 cc. of glacial acetic acid are added bringing the suspension to about PH 5.0. The extract is removed by centrifuging and the residue extracted a second time in the same way.
Residue discarded
Solution saturated with ammonium sulfate and allowed to stand for 6 to 12 hr. The precipitate is collected by filtering through celite in a Buchner funnel with suction.
Precipitate removed from celite and suspended in 500 cc. water and dialyzed through cellophane with stirring against cold running tap water for 24 hr. Solution adjusted to PH 5.0 with acetic acid and filtered through celite
Filtrate Contains some intermedin
Residue washed with 300 cc. distilled water. Filtered as before.
Residue discarded
To the solution is added 1/10th its volume of saturated alcoholic (95) benzoic acid solution and placed in ice box for 1 hr. Filtered by suction through hardened filter paper.
Solute precipitated by pouring into 5 volumes of cold acetone. Filtered and taken up in 100 cc. water and 1/10th volume benzoic acid solution again added. Filtered.
Precipitates freed from benzoic acid by washing with 1 l. cold acetone and extracted 3 times with 150 cc. distilled water.
Solution precipitated with 5 volumes of acetone as before.
Precipitate discarded.
Solution
Precipitate
Solution discarded.
Precipitate dissolved in 450 cc. of extract from benzoic acid and precipitate. Solution adjusted to PH 4.5 and 95 gm. ammonium sulfate added. Centrifuged.
Precipitate taken up in 250 cc. water and 55 gm. ammonium sulfate added.


To combined filtrates 50 gm. ammonium sulfate is added. Filtered through hardened filter paper.
Precipitate is taken up in 100 cc. water. Adjusted to PH 4.5 and 22 gm. ammonium sulfate added.
Solution
Solution 14 gm. ammonium sulfate added.
Precipitate dissolved in 50 cc. water. Adjusted to PH 4.2 and 12 gm. ammonium sulfate added.
Solution
Combined solutions saturated with ammonium sulfate and filtered through hardened filter paper with suction.
Precipitate taken up in 100 cc. water. Adjusted to PH 4.2 and purified as in the previous steps adding the appropriate amounts of salt
Filtrate discarded
Filtered
Precipitate contains LH plus FSH. This fraction may be fractionated as already described above.
The combined precipitates containing the LH are further purified by emulsifying in 50 cc. of water adjusting to PH 4.2 in a 0.2 saturated ammonium sulfate solution. The precipitate is centrifuged off, and the concentration of salt raised to 0.4 saturation. This precipitate contains approximately 40 male R.U. per mg.
Solution contains FSH. It is precipitated by saturation with salt, filtered, taken up in water and dialyzed to remove salt. If tests show the presence of LH, the final fractionation must be repeated.[1]

[1] The FSH preparation may be further purified by precipitating inert material with basic lead acetate. The excess lead is removed by precipitation with bicarbonate. Preparations of FSH have thus been obtained containing between 50 and 75 R.U. per mg.

tate on the other hand is inactive when injected as a picrolonate, but if the picrolonic acid is removed by suitable chemical methods and the material is obtained in the same form as previous to precipitation, the activity is regenerated. It is therefore possible to treat an unfractionated extract with picrolonic acid and obtain a preparation which gives no FSH response but is active for interstitial stimulation and luteinization. If an FSH preparation is similarily treated the precipitate is inactive for follicle stimulation, luteinization and interstitial stimulation. The FSH activity may be regenerated as described above.[3]

Both preparations are definitely protein-like and give all the chemical reactions which would be expected of such material. These reactions are too well known to require listing here.

Methods of Standardization

One of the usual methods for standardizing pituitary gonadotropic extracts is to inject them into immature female rats for a certain period of time (72 to 120 hours) and determine the increase in weight of the ovaries over those of untreated controls. The results so obtained give very little information with regard to the proportion of the two hormones in the extract unless certain other facts are known and taken into consideration. It is possible for a dosage of an extract containing much LH and little FSH to produce the same size ovaries as a dosage of another extract containing much FSH and less LH. The reaction of the stimulated ovaries indicates whether the extract is strongly luteinizing or strongly follicle stimulating but it is impossible to evaluate the exact amounts of FSH and LH between the two extremes. Another factor which must be considered is the length of treatment. When a combination of the two hormones is given, the first response is the growth of follicles, while luteinization does not occur until the follicles have undergone considerable development. Consequently if the ovaries are examined after 3 days of treatment only follicles may be present with very little or no luteinization even though very appreciable quantities of LH may be present in the preparation. Even after 4 days, luteinization may not be extensive, but the follicles are usually large and cystic, and luteinization generally is apparent if LH is present. After 5 days of injection with an unfractionated extract luteinization is far advanced and contrariwise if no luteinization is apparent at this time it is quite safe to conclude that that extract contains little if any LH. As a quantitative assay method for comparing one extract with another, such results are reliable only for pure FSH preparations. The quantitative method used by us for purified FSH preparations is to inject the material in 0.25-cc. doses twice daily for 3 days into 22-day-old rats and weigh the ovaries the morning of the fourth day.

To determine the approximate amounts of the two factors in pituitary tissue or unfractionated extracts we have compared their relative activities by the ovarian and seminal vesicle responses. We have shown (3) that the

[3] Under suitable conditions nitrous acid and formaldehyde inactivate the FSH but do not destroy the activity of LH.

ovarian response is governed largely by the amount of FSH while the seminal vesicle response is determined largely by the LH (9) and even though the response in each case is increased by the presence of the other factor the results give a fair idea of the relative amounts of FSH and LH present. It must be emphasized that the values obtained by this method do not represent the absolute units of either FSH or LH in an unfractionated extract, but we believe they give approximate proportions of the two.

The ovarian test for unfractionated extracts is carried out on rats 22 days old at the beginning of the experiment. They are injected subcutaneously morning and evening with .25 cc. per injection. The ovaries are weighed and examined the morning of the fifth or sixth day. The seminal vesicle test is performed on 22-day-old rats in the same manner with the exception that it is always continued for 5 days and the seminal vesicles together with the coagulation gland weighed the morning of the sixth day. Table 2 gives the analysis of sheep, hog and horse pituitary powder. The horse pituitary preparation produced practically a pure follicular response particularly in the lower dosages, indicating considerably less LH than FSH. This lack of LH was also demonstrated by the response of the seminal vesicles, since it took an amount of powder equivalent to ten times that required for a female rat unit to give a unit response in the male. The hog and sheep powders showed strong luteinizing action and the seminal vesicle response also indicated an abundance of LH as the amount of powder required for a male unit was approximately $\frac{1}{2}$ to $\frac{1}{4}$ of the requirement for a female unit.

Assay of the purified luteinizing hormone may be accomplished in two ways. The first method used by us consisted of recombining the LH with FSH and noting the increase in the weight of the ovaries over that produced by the injection of the FSH alone (3). A R.U. of LH is defined as that amount of the substance which, when combined with 2 R.U. of FSH and injected as described above over a period of 5 days will produce 100% augmentation in ovarian weight. The ovaries must also be clearly luteinized in contradistinction to the follicular development produced by FSH.

A second method involves the use of male rats and the determination of the increase in weight of the seminal vesicles. The unit is taken as that amount which when injected for 5 days twice daily into 22-day-old rats produces 100% increase in the combined weight of the seminal vesicles and the coagulation gland. This is perhaps a more convenient method of standardization since it may be done directly without the use of FSH. The amount of LH required for one male unit is equivalent to about 6 augmentation units.

Saunders and Cole (10) have recently questioned the existence of the luteinizing hormone chiefly on the basis that augmentation of pituitary extracts can be obtained with nonspecific substances. They conclude that the augmentation obtained when LH is added to FSH is merely due to a slowing down of the absorption rate similar to that obtained with tannic acid, or zinc salts. Although this reaction will be dealt with extensively in another paper it should perhaps be given some consideration here inasmuch as aug-

mentation has been mentioned as a possible method for standardizing LH. Data are presented in table 3 showing results obtained when FSH and LH were injected after combination in vitro and when they were injected simultaneously but separately in different subcutaneous areas of the body. Both preparations were prepared in water-soluble form and precipitated with tannic acid, the precipitate emulsified in water and injected as insoluble tannates twice daily for 5 days into 22-day-old rats. The results show that augmentation and luteinization is produced equally well by separate injections as by the injection of the two preparations after combining them in vitro. The augmentation and luteinization produced by the LH is physiological and is

TABLE 3. COMPARATIVE AUGMENTATION OF OVARIAN WEIGHTS WHEN FSH AND LH ARE INJECTED TOGETHER AND WHEN THEY ARE INJECTED SEPARATELY

FSH	LH	Ovarian Weights			Ovarian structures
		Injected together		Injected separately	
mg.	*mg.*	*mg.*	*mg.*	*mg.*	
0.15	0		20		Follicles
0	0.2		14		
0.15	0.1	73		68	C. L.
0.15	0.05	68		66	C. L.
0.15	0.025	40		36	C. L.
0.15	0.012	39		40	C. L. plus F.
0.15	0.006	26		24	Cystic F.

The ovarian weights given are the averages of 3 pairs of ovaries.

not to be confused with increased activity produced by substances which merely decrease absorption rates such as various proteins, tannic acid or zinc salts.

In order to obtain marked augmentation by the combined or simultaneous injection of FSH and LH it is essential that the FSH is free of LH and the LH free of FSH. If the FSH contains LH the response already represents a partial or complete augmentation reaction and the addition of more LH may not result in further increase in ovarian weight, depending on the amount of LH already present. On the other hand, if the LH contains FSH the increased response is due to an additive effect as well as augmentation.

DISCUSSION

It is evident that the methods of assay as given in this paper are based on the belief that the pituitary secretes two separate and distinct gonadotropic substances, FSH and LH. While this belief is accepted by many it is not universal. Saunders and Cole (10) have recently expressed doubt as to the existence of the two factors. With regard to their statements that the physical condition of the hormone, methods of injection and slight chemical changes may account for the differences in the physiological responses obtained with the FSH and LH preparations, we can only reiterate what we have already emphasized (2). Purified FSH and LH preparations are consistent

and distinct in the physiological effects they produce and we have not been able to change their responses by varying any of the above-mentioned factors. The two hormones may possibly be, and probably are, very similar chemically but the difference in chemical structure appears to be definite and so far at least the hormones have not been transformed one into the other in vitro.

The methods for assay of LH are based on the belief that LH is responsible for luteinization and also for interstitial cell stimulation (9). Evans et al. (6) believe that interstitial cell stimulation is the property of a substance which is different from that which produces luteinization, and have reported the separation of such a factor from FSH and LH.[4] We have repeated their separation procedure a number of times and have attempted to fractionate our LH by other methods but have invariably found that any preparation which is active for interstitial cell stimulation in the testes always produces augmentation and luteinization when injected with FSH. The ratio between the dose which constitutes a male unit and that which constitutes a luteinizing unit is constant within experimental accuracy namely approximately 1 to 6. Ammonium sulfate precipitation is of material aid in the separation of FSH and LH but we have not been successful in confirming Evans et al. in obtaining a gonadotropic preparation which does not have the characteristics of either the FSH or LH preparation as we have previously described them. On the basis of our results we do not feel that it is necessary to postulate the existence of two hormones to account for luteinization and interstitial cell stimulation. It is evident, however, that if the interstitial cell stimulating hormone is a separate factor from the luteinizing hormone then seminal vesicle hypertrophy is not a measure of LH but of the interstitial cell stimulating factor.

It seems impossible to standardize a pituitary gonadotropic extract quantitatively for one of the two factors when the other is present. The two gonadotropic hormones are complementary to each other in all physiological reactions which have been used for standardization and while LH, for instance, does not materially increase ovarian weights when given alone, it does have a decided effect when given simultaneously with FSH. On the other hand FSH does increase ovarian weight when given alone and can therefore be

[4] While this paper was in press, a statement by Evans (11) appeared, which is a direct contradiction of earlier reports. In 1937, Evans, Simpson and Pencharz (7) stated: "Injection of combinations of ICSH and FSH gives further evidence that the fraction (ICSH) is free of LH since no corpora lutea are produced under these conditions" and " . . . while FSH contaminated by LH produces tubular repair, it does not repair interstitial tissue." These results would, indeed prove the existence of an interstitial cell stimulating hormone separate and distinct from the luteinizing hormone, discovered and characterized by us in 1931 and subsequently (1, 2, 3, 9). More recently, however, Evans states: "The question of whether, indeed, one is justified in setting up the hypothesis of a special so-called luteinizing hormone is still sub judice, for lutein tissue is readily produced when ICSH, itself incapable of producing lutein tissue, is given concurrently with FSH . . . " (11).

Our characterization of the LH has consistently been that it produces lutein tissue in the female when given simultaneously with FSH or when follicles are already present in the ovaries, and stimulates interstitial cell hypertrophy in the male gonads. Evans and co-workers originally described the postulated ICSH as: "the fraction . . . free of FSH and also of LH as defined in all ways known to us" (7) but now finds that in addition to stimulating the interstitial cells it readily produces lutein tissue when given with FSH (11). From these statements it appears that "ICSH" is now identical with LH and substantiates our reports that interstitial cell stimulation and luteinization are produced by the same substance. Apparently no new substance has been discovered by Evans and co-workers so the term ICSH adds nothing but confusion.

standardized by using ovarian hypertrophy as a measure. This can only be done, however, in the absence of LH. In an entirely analogous manner FSH does not increase seminal vesicle weight but does augment the action of LH in this reaction, consequently this response can be used to assay LH quantitatively, only in the absence of FSH. Comparison of the activities of an unfractionated extract as determined by ovarian and seminal vesicle response as we have done, apparently gives a general idea of the relative proportions of the two in the preparation but the results are not quantitative for either factor. However, it seems to us to be the best method at the present time for comparing such preparations. The only other alternative appears to be a complete separation of the two hormones previous to standardization.

SUMMARY

Methods for the extraction, separation and assay of pituitary follicle stimulating and luteinizing hormones are described. It is pointed out that quantitative assay of the two factors is possible only after complete separation from one another due to the interaction of the two in physiological reactions. An assay method is described however, for unfractionated extracts which gives roughly the relative amounts of each in the preparation.

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THE CLINICAL INTERPRETATION OF THE ENDOMETRIAL BIOPSY: REPORT OF THREE HUNDRED CASES

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THE OVARIAN CYCLE, with the concomitant changes in the endometrium, has been the subject of intensive study during the past decade. In an attempt to clarify some of the points which are still obscure we have studied more than five hundred cases by means of the endometrial biopsy and now report on our findings.

Early descriptions (1, 2, 3, 4) of the endometrium were based entirely upon the morphologic changes encountered, but with the advent of knowledge of the function of the ovarian hormones, these changes can now be interpreted from a physiologic standpoint. Experimental work (5, 6, 7, 8, 9, 10) has demonstrated that the follicular hormone, estrone, produces the early endometrial growth changes characteristic of the first half of the menstrual cycle and that the corpus luteum hormone, progesterone, transforms the endometrium which is primed and maintained by estrone, into a secretory tissue.

Recent investigators (11, 12, 13, 14, 15, 16, 17, 18) have correlated these changes in the endometrium with the estrone-progesterone quantitative changes in the ovaries. These authors do not agree among themselves in the classification of the different phases of the cycle. The first phase has been designated the interval, follicular or proliferative phase and the second has been called the premenstrual, differentiative, secretory or luteal phase. In our study of the endometrial cycle we have corroborated and extended the findings of these authors. While the monthly cycle of endometrial growth does not consist of many well-defined stages but presents an orderly continuous process, for the purpose of identification and as a basis for recognition of pathological changes, certain characteristics of the different phases of development and differentiation may be described.

Normal Endometrial Types

The early follicular phase. During the first week following menstruation, the endometrium is thin and composed of a loose stroma in which few, widely-dispersed, small, simple, straight, tubular glands are present. The cells of these glands are characteristically tall columnar, crowded together and have large dark-staining oval nuclei which occupy all levels of the cells and contain a scanty amount of pale-staining cytoplasm. This crowding of the cells gives them a 'piled-up' appearance. Toward the latter part of this week, mitosis is active and may be seen in both the gland cells and stroma. The stroma cells are spindle-shaped with elongated nuclei and a scanty amount of cytoplasm.

The late follicular phase. Growth of the endometrial elements which has been initiated in the first week of the cycle is continued into the second week, resulting in an increase in both the size and number of the glands. The growth of the stroma is not commensurate with that of the epithelium so that the glands appear more crowded and coiled than those of the earlier phase. There is some dilatation of the lumen of the glands but the epithelium maintains a well-defined even contour in contradistinction to that of the later secretory phase in which it is irregular and ill-defined (fig. 1).

The first half of the endometrial cycle is characteristically one of growth and is dependent upon adequate estrone stimulation. For this endometrium to be transformed into a secretory tissue of normal structure, it must first reach the optimal degree of proliferation. We believe that some of the pathological types of the secretory endometria, to be described later, are due to a failure which dates back to this phase and can not be attributed to faulty corpus luteum function alone.

The post-ovulatory phase. At the height of development of the first phase of the cycle, a spectacular change is apparent in the endometrium which indicates the onset of differentiation (fig. 2).

The first evidence of differentiative changes in the endometrium is the 'ranging into line' (12) of the gland nuclei. The lumen of the gland is therefore increased due to the realignment of the cells and not to the accumulation of secretion, for the cell margins remain clear and well-defined and the cytoplasm is similar to that of the follicular phase. This is rapidly followed by the accumulation of glycogen between the nuclei and the basal wall of the cells which pushes the nuclei to a central position. In stained sections, the glycogen is represented by vacuoles, thus producing the so-called 'sub-nuclear vacuolization' (19, 20) which has been described as diagnostic for the occurrence of ovulation 24 to 72 hours previous to the time at which the specimen was taken (12). However, it has been demonstrated further (21) that large doses of estrin may produce a similar glycogen deposition and migration of the nucleus (22) in the uterine epithelium. These findings make it questionable as to whether subnuclear vacuolization is a good criterion for the occurrence of ovulation in abnormal cycles.

Since ovulation marks the transition from follicular to corpus luteum function, and since the function of these two structures is accurately reflected in the endometrium, it seems reasonable to assume that the first evidence of secretory differentiation in a normal cycle should indicate that ovulation has recently taken place. However, further work is necessary to substantiate this. We are now employing other methods of determining the time of ovulation, such as the appearance of prolan and later of pregnandiol in the urine, which should throw some light on this question.

The early luteal phase. The luteal phase consists of rapid differentiative changes in both the glands and the stroma. Following the phase just described, there is a change in the location of the glycogen from the basal to the peripheral part of the cells which causes all of the nuclei which have now become rounded and pale-staining, to assume a basal position. The glands are then lined with a single layer of cells whose outer margins are well-defined although the cells have begun to contain secretory products. There is also a characteristic change in the stroma which becomes very loose and often edematous. The stromal cells lose their spindle shape, the nuclei becoming rounded and the cytoplasm more abundant. Small coiled arterioles and widely dilated venules extend from the base to the periphery of the endometrium. The arterioles appear to be congested and small hemorrhages are scattered through the superficial layer. In fact, more blood is seen within the tissue at this time than at any other

time of the cycle—a finding which may be significant since this corresponds to the time implantation should occur (fig. 3).

The late luteal phase. At the height of development of the luteal phase, the epithelium reaches its maximum degree of proliferation. The large turgescent cells, filled with very granular cytoplasm are of more or less pyramidal shape with the apex, which bears the nucleus, pointing toward the basal membrane. The outer walls of the cells become attenuated to allow the secretions to escape. The whole epithelial layer becomes so extensive that it is thrown into very characteristic folds on the membrania propria (fig. 4).

Often, but not always, the endometrium can be divided into an outer compact layer, in which the stroma is dense and the gland represented by small round ducts, and an inner spongy layer in which the stroma is scanty, the glands large and close together. In some cases, the stroma of the compact zone presents an entirely different picture at this time, there being an infiltration of a large number of large oval pale-staining cells—the predecidual cells. These predecidual cells may also be seen surrounding blood vessels deeper in the endometrium.

This typical well developed secretory endometrium occurs as the result of the simultaneous action of both of the ovarian hormones. Progesterone is always credited with the production of the differentiative changes, but it must be kept in mind that estrone is produced in greatest quantities—as shown by blood and urine studies—at this time in the cycle and is an important factor in the production and maintenance of this phase. It seems reasonable that pathological types of the endometrium may occur as the result of the disturbance of the normal balance of these hormones at this time.

The phase of tissue loss. Prior to the onset of menstruation, the endometrium may present one of two pictures. One may represent the height of the secretory phase as described above, while the second represents a retrogression of the endometrial elements. In this later type, the glands have discharged their secretions and their lumina are widely dilated while the cells have become low cuboidal. Occasionally the glands appear as strings of flat epithelial cells with pyknotic nuclei. The stroma cells are poorly defined and many have deep-staining small nuclei and no visible cytoplasm. In both types there may be an influx of leucocytes, especially at the periphery of the compact layer. Hemorrhages appear under the epithelial lining of the uterine cavity and along blood vessels.

The termination of the cycle is marked by the rapid exfoliation of both the compact and spongy layers of the endometrium. In a specimen taken after the onset of bleeding, we may see only degenerated tissue, but often well preserved glands, their cells still filled with secretion are discernible. In fact some areas may be intact, showing no evidence of either exfoliation or hemorrhage (fig. 5).

The basal glands of the endometrium, which have not entered into the growth and differentiation of the endometrium are not desquamated. From these the new endometrium is regenerated.

Abnormal Endometrial Types

The occurrences of an atrophic endometrium in the menopause and long standing amenorrhea, and follicular endometrium in pseudomenstruation have been recognized in most recent papers. Follicular hyperplasia, perhaps the best known of the abnormal endometrial patterns, has been described frequently, usually in association with functional bleeding. The literature is very extensive on this subject and has been adequately reviewed (23, 24, 25, 26).

There has been no classification of the abnormalities of the luteal phase which takes into consideration all the types which have been recognized by different investigators. In fact, some authors are of the opinion that the corpus luteum functions in all or none fashion and that once a corpus lutuem has started, it goes through the normal process of development (18). Other authors have expressed the opinion that abnormalities may occur in the luteal phase as well as in the follicular and may be the result of either prolonged, deficient or abnormal corpus luteum function (11, 17).

In reviewing the literature we have found the following types of endometrial abnormalities described as occurring during the luteal phase:

a) Mixed type.
  Follicular and secretary glands (27, 28, 29, 33)
  Follicular stroma with secretory glands (27)
  Follicular glands with secretory stroma (33)
  Interval pattern with secretory glands (32)
b) Prolonged luteal phase—irregular shedding (28, 29)
c) Hypertrophy of the glands during the luteal phase (27)
d) Cystic changes during the luteal phase (17)
e) Poorly developed secretory reaction (31)

METHOD OF STUDY

The menstrual cycles of 500 women were studied by the biopsy method. We were fortunate enough to be able to control 300 of these cases for a sufficient length of time to have accurate data on the menstrual history and adequate biopsy material for study.

The series of cases here presented include *a*), amenorrhea, *b*), oligomenorrhea, *c*), hypomenorrhea, *f*), menorrhagia, *e*), metrorrhagia, *f*), menopause, *g*), sterility, *h*), dysmenorrhea. In each case a complete history with emphasis on the character of the menstrual cycles since the onset at puberty was obtained. General and pelvic examinations were done in each case. Laboratory work consisted of complete blood counts, urinalysis, Kahn, B.M.R. and in most cases blood hormone tests for the estrogenic and gonadotropic substances were obtained at the time of the biopsy.

An endometrical specimen was obtained 2 to 3 days prior to the period. In cases in which there were marked irregularities and this timing was impossible, the biopsy was taken 22 to 26 days after the last period. If the flow did not occur within 5 to 7 days thereafter the biopsy was repeated until one was obtained just prior to the flow, and only the last specimen used for this report. In the case of amenorrhea and those of prolonged bleeding, a biopsy was taken every week for several weeks and the specimen showing the most growth and differentiation was used. Unless consideration is given to the exact time relation of the biopsy and the adjacent periods of bleeding the biopsy is worthless.

We have found that the endometrium does not respond as a whole to the hormonal stimulation. That high up in the uterus usually shows the most advanced and complete growth while that near the cervix may show little or no reaction. There may also be variations with the depth of the endometrium in the fundus. To insure that the biopsy obtained would give an accurate picture we made it a routine to take four downward strokes

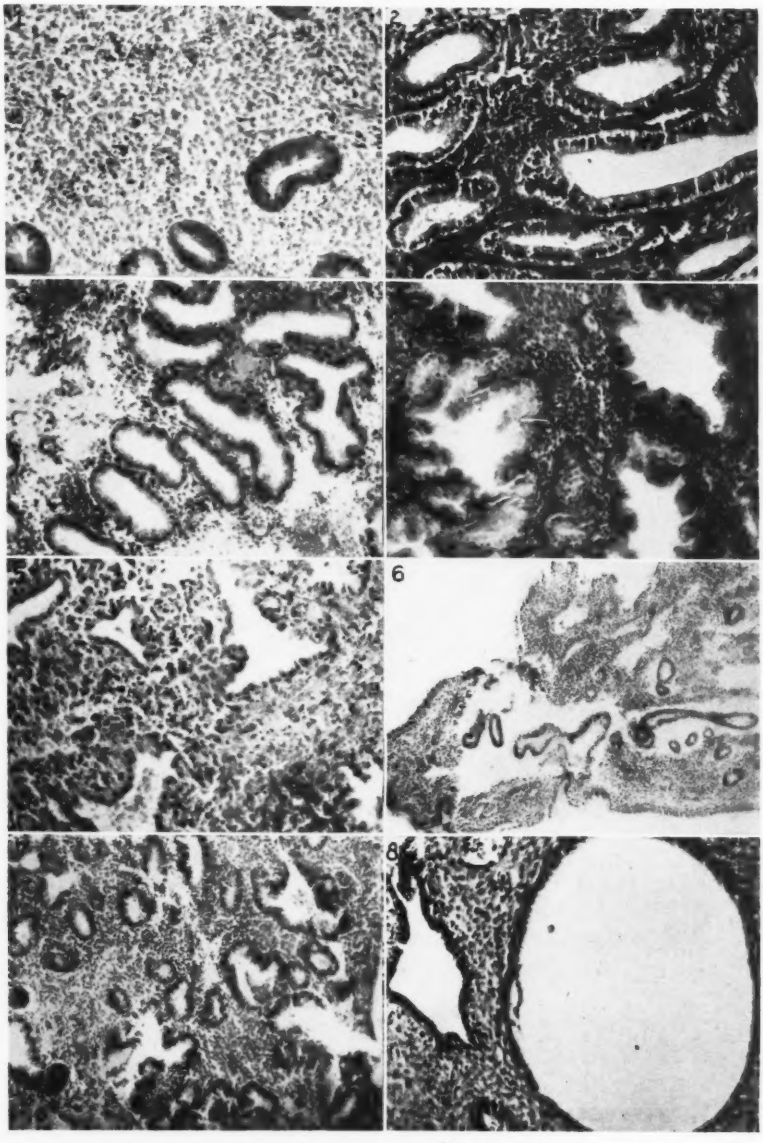

Fig. 1. Follicular phase. Fig. 2. Subnuclear vacuolization. Fig. 3. Early luteal phase. Fig. 4. Late luteal phase. Fig. 5. Phase of tissue loss. Fig. 6. Atrophic endometrium. Fig. 7. Follicular hyperplasia. Fig. 8. Swiss-cheese endometrium. (×130)

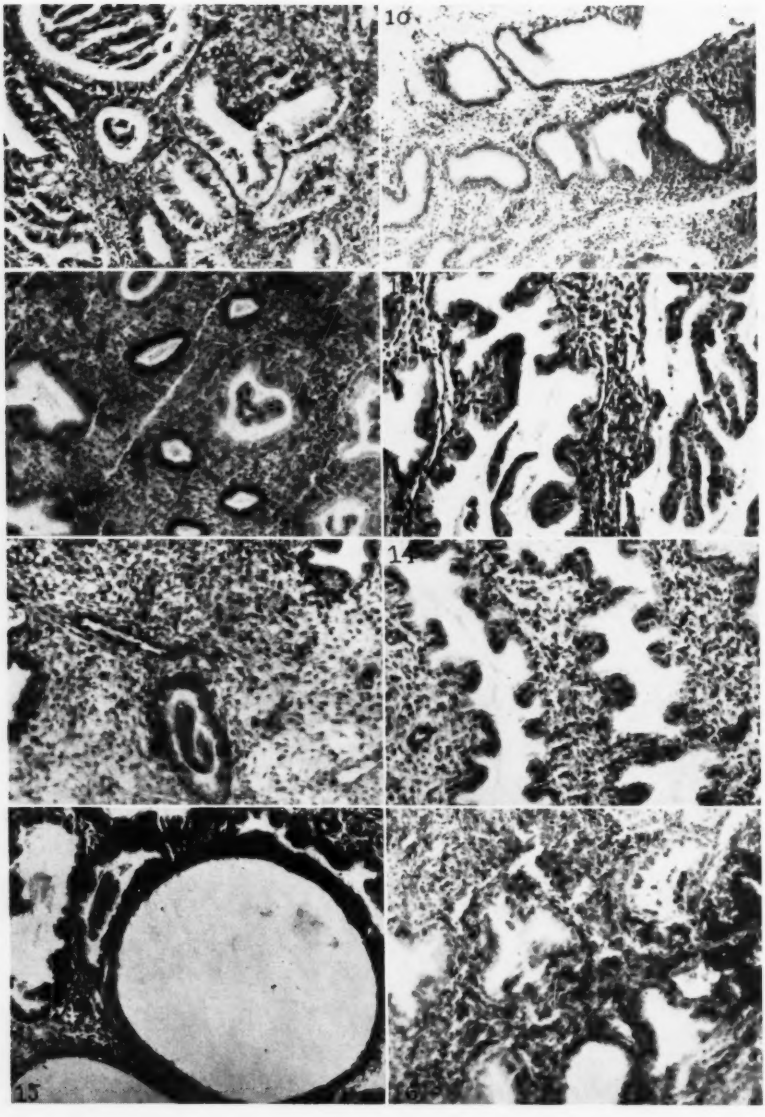

Fig. 9. SUBNUCLEAR VACUOLIZATION WITH FOLLICULAR HYPERPLASIA. Fig. 10. 'LOOSE' TYPE OF LUTEAL PHASE. Fig. 11. 'MIXED' TYPE OF LUTEAL PHASE. Fig. 12. GLANDULAR HYPERPLASIA IN LUTEAL PHASE. Fig. 13. GLANDULAR HYPOPLASIA IN LUTEAL PHASE—Type one. Fig. 14. GLANDULAR HYPOPLASIA IN LUTEAL PHASE —Type two. Fig. 15. CYSTS IN LUTEAL PHASE. Fig. 16. 'IRREGULAR SHEDDING.' (×130)

from as high up in the uterus as possible, two on the anterior wall and two on the posterior wall, being careful to relieve all pressure before the internal os was reached. A Novak suction currette was used.

Specific time limits for the menstrual cycle and its phases were found not to be demonstrable and marked variations were encountered even in the same individual. This is a contrary finding to that of other authors (13). We have accepted as normal a cycle of 25 to 35 days with a 3 to 8-day flow.

In dating a specimen in the follicular phase we count back to the beginning of the last menstrual period. In estimating the date of one in the luteal phase, we count forward to the onset of the next cycle. This is done because the time of ovulation is extremely variable. In most cases where there is a prolongation of the cycle above 28 days, the lengthening occurs in the follicular phase.

RESULTS OF STUDY

An endometrium to be diagnosed as pathological may vary from the normal in one of two ways. The histology of the phase may be apparently normal but its time of occurrence in the menstrual cycle does not conform to that of the normal, producing the delayed or persistent phases, or, the structure of the endometrium may be pathological. Both types represent a disturbance of the hormonal mechanism of the cycle. It is possible that some of these pathological changes may be due to unresponsiveness on the part of the uterus, but we believe this to be of unusual occurrence in this series of cases since the menstrual abnormalities were antedated by a period of normal function of the endometrium and pelvic pathological characteristics were absent at the time of the study. From our study of the endometrium in this series of cases the following endometrial patterns may be described.

Follicular Type

The term 'follicular type' is here used to indicate that the endometrium shows the basic structure of that which is encountered during the first phase of the normal cycle. It does not necessarily refer to the state of the ovarian follicle. However, any endometrium of the follicular type which occurs premenstrually indicates failure of corpus luteum function and usually the absence of ovulation.

Atrophic follicular type. When the endometrium is of this type, often only fragments of the epithelium may be obtained. The depth of the uterus is shallow and the walls feel fibrotic as the scraping is taken. On examination of the tissue, it consists of a loose stroma in which a few small follicular glands are present (fig. 6).

The persistent follicular type. This is a frequently occuring type in which the development of the endometrium is not as advanced as it should be for the time at which the biopsy was taken, the follicular reaction being still present when normally luteal differentiation should have occurred. Such an endometrium does not vary greatly from the usual follicular phase except that there is a tendency for the glands to vary slightly in size, shape, and

general distribution, small and large somewhat dilated glands being irregularly dispersed through the endometrium.

The hyperplastic follicular type. The glands are increased in number and vary considerably in size, shape and distribution. There is also an increase in the number of cells of the glands, which overgrowth causes an increase in the thickness and an infolding of the epithelium often resulting in islands of epithelium within the gland lumen when a cross section of the gland is examined. Mitosis is frequent. The stroma is more dense and the cells spindle shaped. The blood vessels are increased, large thick-walled arterioles and dilated venules are irregularly dispersed through the endometrium (fig. 7).

The cystic follicular type. In such an endometrium, the glands show marked irrgularity in size and shape, many being dilated to the point of cyst formation. The epithelium consists of a single layer of flattened cells. The stroma is dense. Mitosis is usually absent. When such changes are present to a marked degree in the majority of the glands, the result is the Swiss-cheese endometrium (fig. 8).

An atrophic follicular endometrium represents the most profound type of ovarian failure, since in such a case there is not only an absence of corpus luteum function, but a failure of the follicular hormone also. This is probably the most characteristic type of endometrium in that it is always encountered in cases where there has been a cessation of the menstrual function.

That follicular hyperplasia exists has been questioned by some workers (11). But when there is an actual increase in the number of the glands and of the cellular contents of these glands and of the stroma, it seems that the term 'hyperplasia' is entirely appropiate. Since we have found that this condition is often accompanied by high levels of the estrogenic substance in the blood, the correlation of hyperplasia and hyperestrinism seems entirely justified. The term hyperplasia should not be applied to conditions in which the endometrium shows only cystic changes, as is sometimes done. Cystic changes represent retrogression with estrogenic failure and hyperplasia represents overgrowth with excessive estrogenic activity.

In this series, 68 cases of follicular endometria were encountered of which 7 were atrophic, 36 were of the follicular type, 19 were hyperplastic and 6 were of the typical Swiss-cheese type. Forty of these cases in which the specimen was taken during bleeding or was followed immediately by bleeding could be classified as pseudomenstruation. In 12 cases in which the biopsy was followed by bleeding in 5 to 7 days we considered that it represented persistence of the follicular phase but not necessarily pseudomenstruation, since some luteal activity might have intervened in the time between the biopsy and the bleeding, although it would have been of the abnormal type. The remaining 16 cases were of amenorrhea, oligomenorrhea or menopause.

The Delayed Post-Ovulatory Type

We have elected to call the endometrium in which the subnuclear vacuolization is the predominant characteristic the post-ovulatory type. This type may occur as one of three variations namely: The structure of the

endometrium may be normal but this phenomenon is present at the onset of menstruation, which according to the criterion set forth above is distinctly abnormal. Secondly, subnuclear vacuolization may be superimposed upon a cystic follicular type of endometrium, in which case the type and arrangement of the glands is similar to that described above with this early secretory differentiation present in a good portion of the cells. It is rarely of as uniform distribution as it is in the normal. Thirdly, these differentiative changes may occur in glands which show follicular hyperplastic changes (fig. 9).

It has usually been said that ovulation occurs at the end of the second week of the cycle. If subnuclear vacuolization is a criterion of ovulation within the previous 24 to 72 hours, our data tends to show that the time of

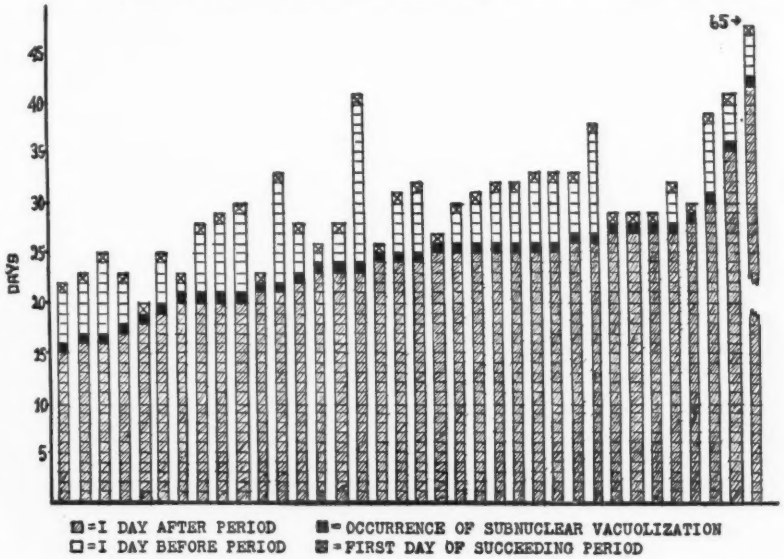

FIG. 17. THE TIME OF OCCURRENCE OF SUBNUCLEAR VACUOLIZATION IN THE MENSTRUAL CYCLE—Thirty-six Cases.

ovulation varies considerably (fig. 17). The earliest time at which subnuclear vacuolization was encountered was 16 days after the onset of the last menstrual cycle, the longest time which elapsed was 60 days, while the average for the group was 26 days. The total length of the cycle varied from 19 to 65 days with an average of 30.5 days. Biopsy was followed immediately by flow in 4 cases and on the next day in 3 cases which were also the expected times for the periods. The flow was delayed 10 days in one case, 11 days in two cases and 17 days in one case. The average length of time elapsing between the biopsy and the flow was 5 days.

Of the 36 cases showing subnuclear vacuolization, 21 endometria were apparently normal in structure, while 15 showed evidence of cystic or hyperplastic changes having occurred during the follicular phase. The blood

hormone tests showed a low level of the estrogenic substances in the majority of cases and no cases of excess of the gonadotropic substance.

Normal Secretory or Luteal Phase Endometria

There were 90 cases which conformed to the previously described normal secretory reaction, 58 of the early type and 32 of the late. In the group exhibiting the early secretory reaction the time which had elapsed since the last period varied from 20 days to 4 months, the average being 25 days. The succeeding flow occurred in from 1 to 6 days, the average being 3 days. In those exhibiting a late secretory reaction the time which had elapsed since the last period was 23 to 44 days with an average of 27 days and the succeeding flow occurred in from 1 to 6 days with an average of 2 days. All blood hormone tests yielded normal results.

These data bring out several points of interest: first, an apparently normal secretory endometrium can be developed after four months of amenorrhea; second, while in this group of cases, the average figures correspond to the accepted normal, there is a tremendous variation in the time element in individual cases; third, bleeding may occur from endometria exhibiting varying degrees of secretory differentiation; and fourth, once a secretory reaction has developed, uterine bleeding always follows shortly thereafter.

Abnormal Luteal Endometria

The diagnosis of pathological changes in a secretory endometrium is difficult. There are probably changes which are abnormal and which we are unable to recognize as such at present because we do not know the optimal balance and the endometrial response of the ovarian hormones which are maintained at possibly varying levels for one to two weeks of the menstrual cycle. The following deviations from the normal we have interpreted as distinctly pathological from the functional aspect.

Loose or spongy secretory type. In this type, the stroma remains extremely loose and edematous throughout the cycle—as it is early in the secretory phase. The epithelial cells never attain the high columnar type or show the secretory activity as described for the normal. The tissue cannot be divided into compact and spongy layers. Decidual cells are rare. The glands appear to be tubular with a marked dilatation of the lumen. There is little or no infolding of the epithelium which just prior to the onset of menstruation becomes extremely flattened. That this is not just a developmental phase is demonstrated by the fact that these characteristics may be identified at any time of the luteal phase if differences attributable to age are taken into account. In structure, this endometrium gives the impression of a persistence of the hormonal balance characteristic of the very early secretory phase (fig. 10).

This loose or spongy type of endometrium occurred in 32 cases. The biopsy was taken in from 19 to 37 days after the last period and on the average of 25 days. Bleeding followed in from 1 to 9 days with an average of 3½ days. The blood estrogenic substance varied from low to excess while there were occassional occurrences of excess gonadotropic substance.

Mixed type of endometria. The term 'mixed endometrium' is used to indicate the simultaneous occurrence of follicular and luteal characteristics in the endometrium. A certain degree of mixture in the reaction of the endometrium is physiological, as has been pointed out previously. This is especially true during the early secretory phase when fully 50 per cent of the cases here presented might have been called mixed endometria if consideration was not also given to the time element and the normal appearance of the tissue as a whole.

A mixed endometrium of an abnormal type may be represented by any combination of follicular and luteal characteristics of gland and stroma. The most common type is that in which areas of follicular glands and stroma occur in combination with secretory glands and stroma, but secretory glands in follicular stroma and follicular glands with secretory stroma may occur. The mixture of reaction may apply to cells of the same gland, to adjacent glands in the same area or to irregularly distributed areas. In most cases with a mixed pattern other abnormalities are usually evident. The endometrium as a whole appears to be poorly developed, and the areas of variation are irregular and patchy. The secretory reaction is usually incomplete. Hyperplasia and cysts are common. A diagnosis of a mixed endometrium, should not be based on the occurrence of a single small area of variation but the endometrial pattern as a whole should be that of a mixture (fig. 11).

In this series of cases 23 endometria were of the mixed type. The biopsy was taken 16 to 28 days after the last period, the average day being 20. The succeeding period occurred in 1 to 4 days, the average being 2 days. The blood estrogenic substance tended to be low, and the gonadotropic substance was not demonstrable.

Glandular hyperplasia in the luteal phase. The endometria of 5 cases had distinct characteristics which we have interpreted to indicate hyperplasia. The glands were larger than normal with excessive proliferation of the epithelium which resulted in marked and rather bizarre infolding of the epithelium. The epithelial cells were of the luteal type, of a single layer but the secretory reaction was less than normal. The stroma was more dense than usual. Such a reaction, though not of uniform distribution was the predominant characteristic of the endometria. Such hyperplastic changes but present to a lesser degree were also encountered in the mixed types (fig. 12).

The specimens were obtained 22 to 28 days after the last period the average being 24 days and the succeeding flow occurred in 1 to 5 days, on the average of 2 days. The estrogenic substance tended to be excessive and the gonadotropic substance was not demonstrable.

Glandular hypoplasia in the luteal phase. The endometrium was poorly developed in 22 cases which we have interpreted as a condition of hypoplasia. There were two types. In the first, the glands which were fewer in number than normal, were small and showed very little secretory reaction. The stroma appeared to be in a stage of differentiation between that of the follicular and the secretory. Cysts were common. In the second type, the

glands were of normal number, but small, proliferation and secretory differentiation both appearing to be deficient (fig. 13, 14).

The specimens were obtained 16 to 120 days after the last period, the average being 26 days, and the succeeding flow occurred in from 1 to 3 days and on the average of 2 days. Estrogenic substance tended to be low and gonadotropic substance was not demonstrable.

The true significance of the endometrial patterns of the luteal phase, here described as abnormal, will be apparent only when they have been correlated with the actual functional levels of the ovarian hormones. At the present, the interpretation of these types can be made only upon the basis of the known action of these hormones.

The 'loose' secretory type of endometrium, by its structure, would seem to indicate that the secretory differentiation has been arrested at an early stage and that the hormone balance characteristic of the early luteal phase has persisted through the remainder of the cycle.

Glandular hyperplasia probably represents a relative excess of estrone action in the luteal phase. It is known that this hormone is responsible for the proliferative changes of the endometrium. In this type of abnormality proliferation is exaggerated, whereas the secretory changes which occur as the result of progesterone action are present in a minimum degree.

Glandular hypoplasia represents a failure of growth and differentiation. In the first type described, it would appear that a deficient luteal action followed a deficient follicular function, while in the second type, failure appeared to have occurred only in the luteal phase. In both types the luteal failure represents a deficiency of both hormones.

A mixed type of endometrium represents another type of partial corpus luteum failure in which there is primarily a deficiency of progesterone. But it seems possible that it may also represent previous low follicular function in which the endometrium was not adequately primed for progesterone action by estrone.

Cystic Endometrium

Cystic dilation of the glands has been recognized in every type of endometrium in this series of cases. The occurrence in the follicular type has been noted previously. Cysts were encountered in 30 cases of the secretory type. They occurred in both the normal and the abnormal but were more frequent and more numerous in the latter. There was no instance where cystic changes in the secretory type of endometrium was the characteristic finding. We do not feel therefore that cysts in a secretory endometrium represent a specific pathologic entity. They represent an abnormality which should be interpreted in terms of the basic endometrial pattern (fig. 15).

The Stage of Tissue Loss

All types of the endometria except the follicular atrophic were encountered in those cases in which the biopsy was taken during the period of bleeding. Those of a follicular and post-ovulatory type have been previously noted. There were 24 cases in which bleeding occurred from a secretory

endometrium. The pattern of the endometrium varied from a well developed and maintained secretory reaction to one of complete secretory exhaustion. Instances of mixed, hyperplastic and hypoplastic types also occurred. There were 3 cases which demonstrated a well developed luteal phase who had been bleeding for 4 to 6 weeks—a finding similar to that which has been previously described as 'irregular shedding' (28). The structure of these three endometria was not different from that of other bleeding secretory endometria (fig. 16).

The Relation of Endometrial Findings to Gynecological Symptoms

The association of the different types of endometria with different symptoms is summarized in table 1. It is quite apparent that there are no specific

TABLE 1. THE RELATION OF ENDOMETRIAL PATTERNS TO GYNECOLOGICAL SYMPTOMS

	Amenorrhea	Oligomenorrhea	Polymenorrhea	Sterility	Dysmenorrhea	Menopause
FOLLICULAR						
Atrophic	2	0	0	0	0	5
Persistent	3	14	17	8	7	2
Hyperplastic & Cystic	0	8	25	0	0	4
POST-OVULATORY						
Normal	0	12	4	5	3	0
Hyperplastic	0	5	10	2	2	0
LUTEAL						
Normal	0	35	9	18	11	0
Loose	0	11	4	15	6	4
Mixed	0	5	4	8	7	3
Hyperplastic	0	4	2	1	2	0
Hypoplastic	0	6	7	7	3	4
TOTALS	5	100	82	64	41	22

endometrial patterns for the menstrual disorders. Amenorrhea may possibly be an exception since here the endometrium is usually in a stage of follicular arrest or atrophy. When other types have occurred, menstruation has followed shortly thereafter.

Specific changes in cases of menopause were not demonstrable but the endometrium merely reflected the degree of ovarian failure. In early menopause, an abnormal luteal phase was found, later with further loss of ovarian function, the follicular and eventually the atrophic phases occurred. Cysts were encountered but they were not found to be present in all cases of menopause or to be limited to cases of the menopause.

Oligomenorrhea and polymenorrhea occurred with practically every type of endometrium, but with significant variations in distribution as to type. In the cases of scanty and irregular periods (grouped together as oligomenorrhea) 78 per cent showed evidence in the endometrium of some luteal function, although in 38 per cent it was of an abnormal type. In the cases of functional bleeding or polymenorrhea 51 per cent showed complete lack of corpus luteum function while an additional 33 per cent showed abnormal

corpus luteum function as evidenced by the endometrial pattern. The significance of these findings is not apparent. The actual cause of cyclic uterine change is not known. The occurrence of bleeding from all phases of the endometrial development with varying levels of the ovarian hormones would seem to suggest that a third factor—which has been called the 'bleeding factor' (30) is a necessary activator of this phase of the cycle (37).

Sterility is usually due to more than one cause and may be associated with any type of abnormality of the menstrual flow. We have found in a recent study of sterility (34) that when the only cause of the sterility is of endocrine origin, the failure is usually profound, being evidenced by a low basal metabolic rate, infantile uterus and pseudomenstruation.

Dysmenorrhea is a subjective symptom and may also be associated with any type of abnormality of menstrual flow. In the purely functional type of dysmenorrhea we have found that there is usually a relative and often actual preponderance of the action of estrone (35, 36).

The ovarian-endometrial cycle is a labile phenomenon, in which a definite sequence of events normally occurs but to which specific time limits cannot be attached. Any disturbance in the normal balance of the ovarian hormones is reflected in the structure of the endometrium. For this reason the endometrial biopsy, carefully performed and adequately controlled, is the most efficient and simplest method which we have at the present time of determining ovarian hormone function. By such studies, it has been shown that there is no correlation between the type of endometrium and the disturbance of the menstrual flow.

SUMMARY

Three hundred endometrial biopsies from completely controlled cases in which ovarian dysfunction was suspected have been studied and classified. Abnormalities, which occurred in every stage of development, have been described and interpreted in terms of disturbance of the functional level of the ovarian hormones.

Abnormalities of the endometrium reflect either failure of orderly elaboration or a disturbance in the normal balance of the two ovarian hormones at various times in the menstrual cycle. Pseudomenstruation which is often considered to be synonymous with anovulatory menstruation is not uncommon, occurring in 40 cases of this series.

Subnuclear vacuolization which has been described as diagnostic of the occurrence of ovulation is exceedingly variable in time of occurrence and may be superimposed upon an abnormal follicular type of endometrium. Secretory differentiation may be instituted in an endometrium which shows evidence of either deficient or excessive follicular proliferation. The corpus luteum does not function in an 'all or none' fashion but may elaborate an excess of one hormone (estrone) or a deficiency of one or both hormones.

There are no specific endometrial patterns for the gynecological symptoms, with the possible exception of amenorrhea in which a follicular endometrium is usually encountered. Pathological bleeding may occur from any

type of endometrium described. These same types occur in cases in which there is no tendency to bleed. Menopause has no specific endometrial pattern. The endometrium merely reflects the degree of ovarian failure.

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EFFECTS UPON UTERINE MOTILITY OF URINE EXCRETED DURING NORMAL LABOR, NORMAL MENSTRUATION AND DYSMENORRHEA[1]

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IN A PREVIOUS STUDY (1), it was found: *a*), that human urine at times contains a substance or substances which affect the motility of the rabbit uterus, *b*), that certain variations in the effects of the urine occur during menstruation, and *c*), that the variations associated with essential dysmenorrhea are different from those associated with normal menstruation. Since the pain in essential dysmenorrhea appears to be due to uterine contraction, it seemed wise to compare the effects of urine excreted during normal labor with the effects of that excreted during dysmenorrhea. Accordingly, the present study was undertaken. It consists of an investigation of the action upon uterine motility of urine excreted during late pregnancy and labor and a comparison of the results obtained with those of the previous study (1).

MATERIALS AND METHODS

Fifty specimens of urine were examined; they were obtained from 33 women who experienced normal labor. In 30 cases the urine was excreted during the last 57 days of pregnancy and in 20 cases it was excreted during labor. Twenty-three patients furnished one specimen each; 10 patients, 2 to 4 specimens each. Specimens from the latter group were obtained at intervals before the onset of labor (3 cases), during the first and second stages of labor (1 case) or before and during labor (6 cases). Seventeen of the specimens were obtained by catheterization, the remainder were voided.

The urine was tested for its effect upon the motility of the rabbit uterus in situ. The procedure followed was identical with that used in studying the action of urine from non-pregnant individuals (1). The test animals were mature, unanesthetized, castrated does which had been injected with estrin[2] 24 to 48 hours previously (2). Kymograph records of the uterine motility were obtained by means of an intrauterine balloon inserted through a uterine fistula (3) and connected to a tambour through a water and air transmitting system. After a preliminary record of the estrin-induced motility had been obtained, the urine to be tested was injected into the marginal ear vein.

[1] This work has been aided by a grant to Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

[2] Progynon-B, Schering Corporation.

Recording was continued for 30 minutes, then 0.2 cc. of pituitrin[3] was injected intravenously. In all, 69 tests were performed.[4] The dosage of urine was regulated by the response produced, the maximum dose being 20 cc.

RESULTS

Of the 50 urine specimens examined, 40 produced marked responses of the uterus, 8 produced insignificant variations in the uterine motility and 2 had no demonstrable effect. The action of the urine was almost instantaneous, the response beginning, as a rule, during the injection. The responses obtained were similar in type to those obtained with urine from non-pregnant individuals. Nineteen of the 40 positive specimens produced responses in which an initial phase of contraction was followed by a phase of inhibition during which the rhythmical motility was reduced in magnitude, as compared

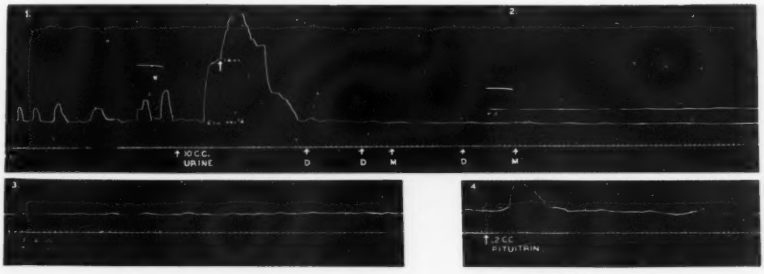

Fig. 1. Response to urine in which an initial phase of contraction was followed by a phase of inhibition. M, Micturition; D, Defecation.

with that present before the injection of the urine, and the sensitivity of the uterus to pituitrin was decreased (fig. 1). Twenty specimens produced responses in which the inhibitory phase was predominant, the contraction phase absent or insignificant in magnitude (fig. 2). One specimen produced a response in which only the contraction phase was present (fig. 3).

The effects of the urine in relation to the onset of labor are shown in the accompanying table. For comparison, the results previously obtained with urine from non-pregnant individuals (1) are included in the table. A comparison of all the groups included in the table shows that the per cent of specimens producing inhibitory effects, considered separately, was highest in the case of urine excreted during late pregnancy and labor or during normal menstruation, and lowest in the case of urine excreted during dysmenorrhea. The per cent of specimens producing contraction, considered separately, was highest in the case of urine excreted during dysmenorrhea. Responses in which the contraction phase was predominant were produced in significant numbers only by urine from women with dysmenorrhea. No responses of this type were produced by urine excreted later than 39 days before the onset of labor. On the other hand, no responses in which the inhibitory phase

[3] Pituitrin-O, Parke, Davis & Company.

[4] Nineteen specimens were tested twice. In 15 instances, pituitrin was injected 15 minutes after the injection of urine in one of the two tests.

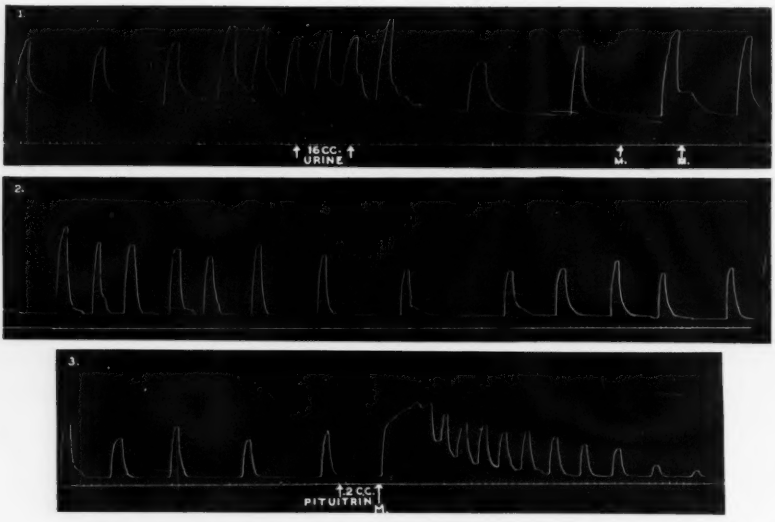

Fig. 2. Response to urine in which only the inhibitory phase was present. M, Micturition.

was predominant were produced by urine excreted during dysmenorrhea or by urine from men. Twenty (55.5%) of the 36 intermenstrual specimens from women with dysmenorrhea produced responses in which one phase was predominant, whereas only 5 (20%) of the 25 intermenstrual specimens from women with no cramps and only 1 (12.5%) of the 8 specimens from men produced responses of this type.

DISCUSSION

These results indicate that the inhibitory activity of urine excreted during the last 30 days of pregnancy and during labor is markedly increased,

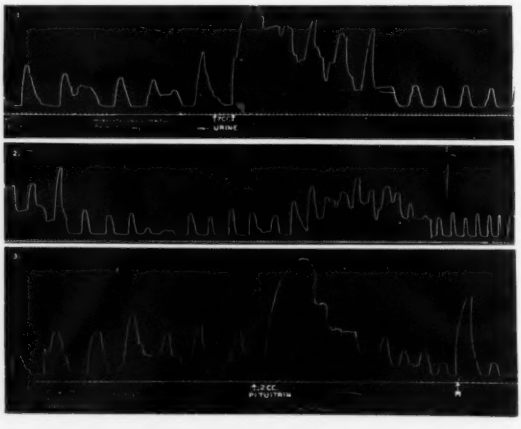

Fig. 3. Response to urine in which only the contraction phase was present. M, Micturition.

as compared with the activity of urine excreted during the normal intermenstruum. During normal menstruation, there is a similar increase in the inhibitory activity. During dysmenorrhea, on the other hand, there is a decrease in the inhibitory activity of the urine and a marked increase in the stimulative activity. Apparently dysmenorrhea is the only one of the conditions investigated which is associated with variations of this type. The results indicate further that, with respect to the excretion of substances influencing uterine motility, there is a variation from the normal during the intermenstruum as well as during menstruation in women with dysmenorrhea.

TABLE 1. EFFECTS OF URINE UPON UTERINE MOTILITY IN RELATION TO THE CONDITIONS PRESENT AT THE TIME OF EXCRETION

	Pregnant women				Non-pregnant women							Normal men	Totals
					No cramps		Mild cramps[2]		Dysmenorrhea[3]				
	Days before Delivery			In labor					Menstruation				
	33–37	16–30	1–13		Menstruation	Intermenstruum	Menstruation	Intermenstruum	Severe cramps	Before or after cramps	Intermenstruum		
No. Individuals represented	5	11	11	15	12	8	9	7	11	11	12	8	70
No. Specimens investigated	6	12	12	20	20	25	20	30	15	19	36	8	223
Per cent of all specimens in each group producing Responses in which	Percentages of all urine specimens in each group giving responses listed												
Both phases present	33.3	33.3	50.0	35.0	35.0	36.0	45.0	40.0	33.3	26.3	22.2	50.0	
Contraction predominant	16.6	00.0	00.0	00.0	10.0	12.0	10.0	10.0	66.6	42.1	27.7	12.5	
Inhibition predominant	00.0	41.7	50.0	45.0	35.0	8.0	15.0	23.3	00.0	00.0	27.7	00.0	
Insignificant responses	50.0	8.3	00.0	20.0	10.0	32.0	15.0	13.3	00.0	26.3	16.6	25.0	
No demonstrable effect	00.0	16.6	00.0	00.0	10.0	12.0	15.0	13.3	00.0	5.3	5.5	12.5	
Contraction[1]	50.0	33.3	50.0	35.0	45.0	48.0	55.0	50.0	100.0	68.4	50.0	62.5	
Inhibition[1]	33.3	75.0	100.0	80.0	70.0	44.0	60.0	63.3	33.3	26.3	50.0	50.0	

[1] Considered separatedly.
[2] Menstrual cramps which did not interfere with normal activity.
[3] Menstrual cramps of incapacitating severity.

It is generally agreed that uterine contraction is the immediate cause of the pain in dysmenorrhea. The nature of menstrual pain itself furnishes almost incontrovertible evidence in favor of this view. The assumption is still further supported by the fact that the motility of the human nonpregnant uterus reaches its maximum just before the onset of menstruation and during the first days of the menstrual flow (4, 5, 6). Satisfactory evidence of the occurrence of abnormal contractions in women with dysmenorrhea has not been obtained (6), although it has been observed in a few cases that the contractions were more forceful (5, 7) or more rapid (8) than those observed in normally menstruating women. Adair and Davis (9), who studied the relationship of pain to uterine contraction in the period immediately post partum, found that the difference between painful and painless contractions was in frequency and amplitude, not in type. Moir (5) has suggested that menstrual pain may be due to ischemia and that its presence or

absence may be determined by the relationship existing between the force of the uterine contractions and the blood pressure of the patient. In this connection, it is of interest that Lubin, Clarke and Reynolds (10) found that after-pains could be relieved by the administration of progesterone and that relief was experienced prior to the appearance of any significant alteration in the record of the uterine contractions. Lackner, Krohn and Soskin (11) made similar observations in two cases of dysmenorrhea treated with progesterone; in three other cases they observed a decrease in the degree of uterine motility coincident with the disappearance of pain.

As regards the underlying cause of dysmenorrhea, certain possibilities have been eliminated and certain suggestive evidence has been obtained as a result of extensive clinical investigation. It has been shown that no one of the local abnormalities (anatomic anomalies, clots, menstrual membrane) which have been suggested as causes is found in all cases of dysmenorrhea, all of them are absent in many cases, and each of them occurs in women who menstruate without pain. Hence the weight of the available evidence is against the conception that dysmenorrhea is due fundamentally to stimuli of a mechanical nature which result from the presence of some local abnormality. Likewise, clinical experience has shown that theories attributing dysmenorrhea to disorders of the central nervous system are wholly inadequate to explain the occurrence of menstrual pain in general, although psychic trauma or abnormal sensitivity undoubtedly plays a part in some cases. On the positive side, considerable evidence which suggests that dysmenorrhea is due to an endocrine disturbance of some type has accumulated.

Analysis of the findings thus briefly summarized justifies the assumption that menstrual pain results from a physiologic disorder, probably endocrine in nature, which gives rise to a derangement of the uterine motility normally associated with the onset of menstruation. Recently a number of theories based on this concept of dysmenorrhea have been advanced (12, 13). These theories postulate abnormalities in the endocrine activity of the ovary. They are in harmony with present knowledge of uterine physiology but no direct evidence in support of them could be found in the literature (11, 14, 15). In so far as can be determined by the criteria used at the present time, there is no constant histologic change in the endometrium common to all cases of dysmenorrhea, although certain changes possibly indicative of various degrees of change in ovarian function have been observed in some cases (16).

In the present study, evidence has been obtained that certain variations in the composition of the urine occur during menstruation and during late pregnancy and labor. The variations found to be associated with normal menstruation and with labor, conditions which are characterized by the occurrence of normal uterine contraction, were similar, whereas those associated with dysmenorrhea were of an entirely different type. Moreover, a marked imbalance in the excretion, during the intermenstruum, of substances influencing uterine motility was observed in women with dysmenorrhea, whereas comparatively little imbalance during the intermenstruum was ob-

served in normal women. Since variations in the composition of the urine are dependent upon variations in physiologic processes, these observations indicate that there is some deviation from normal in the physiologic processes associated with painful menstruation and that there is a certain degree of physiologic imbalance during the intermenstruum in women with dysmenorrhea. In other words, the results of these experiments afford direct evidence of the presence of a physiologic abnormality in women who suffer from dysmenorrhea. They are consistent with the view that this abnormality gives rise to disordered uterine contraction, although they do not, of course, necessarily imply any causal relationship between substances in the urine and the type or degree of uterine motility associated with their excretion.

SUMMARY

Urine excreted during the last 57 days of pregnancy and during labor was examined for its action upon the motility of the rabbit uterus in situ and the action of this urine upon uterine motility was compared with that of urine excreted during menstrual and intermenstrual phases of the cycle by normally menstruating women and by women with essential dysmenorrhea. It was found that urine excreted during the last 30 days of pregnancy and during labor shows an increase in inhibitory activity, as compared with urine excreted during the normal intermenstruum. Urine excreted during normal menstruation shows a similar increase in inhibitory activity. Urine excreted during dysmenorrhea, on the other hand, shows an increase in stimulative activity and a decrease in inhibitory activity. Further, women with dysmenorrhea, in contrast to normally menstruating women, exhibit a marked imbalance in the excretion during the intermenstruum of substances which influence uterine motility. These observations indicate that:

a) The physiological changes associated with labor and with normal menstruation are similar, insofar as they affect the excretion in the urine of substances which influence the motility of the rabbit uterus.

b), There is a disturbance in the physiologic processes of women who suffer from dysmenorrhea and this gives rise to characteristic variations from the normal in the excretion in the urine of substances which influence the motility of the rabbit uterus.

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THE TECHNIC OF HYPOPHYSECTOMY IN THE RABBIT

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SMITH AND WHITE (1), who first used this operation, did not describe it, but later White (2) indicated the successive steps quite clearly. The present author believes that, in spite of this, most people experience considerable initial difficulty which can easily be overcome by suitable equipment and attention to certain details. The rabbit is a notoriously bad operative risk, the field is cramped and everywhere prone to hemorrhage, adequate illumination is difficult and binocular vision cannot be maintained throughout. No apology is, therefore, necessary for mentioning details which might otherwise be taken for granted. Those who are successfully practising the operation must already have solved the difficulties in their own way, but it is hoped that this full account will be helpful to others. It is based on about 50 operations in which success became more certain as various small modifications were introduced, and is intended to supplement White's description, which should be read first. The time taken is much the same as stated by White, the alterations tending to hasten the approach and lengthen the time spent in the pituitary fossa.

PROCEDURE

Anesthetic. As the operation is in the respiratory tract, the shorter the length of time the animal remains unconscious after the operation the better. Ether is therefore the anesthetic of choice, and, after induction, is led in a stream of oxygen through a tube attached to the tongue retractor deep into the animal's mouth. Alone, it is unsatisfactory, the depth of anesthesia altering rapidly, and the respiration being too irregular to act as a guide. A subcutaneous injection of 2.4 cc. per kg. of body weight of a 25 per cent solution of magnesium sulphate half an hour before induction transforms ether and oxygen into an almost perfect anesthetic. The respiratory movements are quiet and regular, and their vigor is a reliable indication of the state of anesthesia. Atropine is unnecessary; there may be some accumulation of secretion in the first 5 minutes of the operation, but this is easily cleared and does not recur. The animal is moving about on its feet within 30 minutes of the withdrawal of ether.

Fixation of the Head. A board similar to that used by White was made, and need not be further described. The animal lies on its back with the head clamped laterally, the jaws stretched open, and the cheeks and tongue retracted.

The Soft Palate. A pair of fine forceps, splayed out at the points (fig. 1), is inserted into the mouth, placed closed on the soft palate, and released. The points catch in the mucosa (without penetrating it) and render the palate taut from side to side. The upper part of the forceps rests against the upper incisor teeth. The palate is incised longitudinally, exactly in the mid-line, and layer by layer. There is very little hemorrhage, and this can be arrested

Photo by F. J. Pittock

Fig. 1. From above downwards: suction tube for sella (1.5 mm.); suction tube for use during incision of sphenoid mucosa; bone wax syringe (1.25 mm.); palate retractor; dental plastic instrument (2 & 3 mm.); Bowman's needle (2 mm.); spoon for removing pituitary (2.8 mm.); elongated burr (2.25 mm.); suction tube for clearing pharynx of blood and secretion. The dimensions in brackets give the actual outside diameters at the ends.

quickly by shreds of cotton wool dipped in 1/1000 adrenalin. The upper fibrous layer of the palate is not incised until the bleeding, if it occurs, has practically stopped. Blood running through into the deep nasopharynx at this stage may create an emergency. The incision stretches from the thin posterior part of the soft palate anteriorly, the whole length of the fleshy portion, almost to the hard palate, and is 1 to 1.5 cm. in length. On no account is the knife carried against the hard palate, or the soft palate undercut at its anterior end; there is a large vessel, or extremely vascular mucosa here, which gives rise to violent hemorrhage. The forceps is now removed and re-inserted with the splayed points deep to the fibrous layer of the palate. This retracts the palate, and if the forceps is of the right size (fig. 1) the upper part can be pressed over the incisor teeth, increasing the retraction and stabilising

the instrument. With the other instruments as described below, this does not get in the way, takes but a moment to adjust, and does not slip.

Illumination. The narrow nasopharynx is now exposed, about half an inch deep and, towards the roof, one eighth of an inch wide. As the pituitary is approached, the cleft becomes deeper. A headlamp does not solve the problem of illumination, because binocular vision is impossible in the final stages, and sometimes it is convenient to use one eye, sometimes the other. The best results have been obtained with a small lamp, containing a 6-volt bulb, removed from a headpiece and mounted on the end of a piece of flexible 'compo' tubing. It is placed over the animal's mouth slightly to the left side and at a convenient height so that it shines into the wound with its rays approximately parallel to the tongue retractor. If properly adjusted it will not interfere in the least with the subsequent course of the operation. Visibility is materially enhanced by darkening the room, focussing down the area of illumination, and using a black sterile cover instead of the usual white cloths. Magnifying spectacles, etc. are a matter of individual preference, but are not necessary.

Exposure of the Sphenoid. The mucosa covering the roof of the nasopharynx is spongy, extremely vascular, and conceals a vein running longitudinally. The merest scratch results in an astonishing amount of hemorrhage, sufficient to fill the nasopharynx and cause inhalation of blood. White recommends de-vascularising the mucosa by pressure for 15 to 20 minutes. Whatever method is used, incomplete devascularisation necessitates the hurried introduction of cotton wool or gauze, and this tends to abrade the mucosa of the lateral walls which then ooze blood persistently. A blob of silver nitrate fused on the end of a piece of silver wire is an efficient cautery, and allows a somewhat quicker approach, but this also tends to cause rather extensive local damage. The following procedure has been found the safest and quickest. A glass tube is drawn out and shaped so as to fit beside the tongue retractor (fig. 1) and enter the nasopharynx through the posterior angle of the palate incision. It is adjusted, preferably by a rack and pinion, so that its orifice is a millimeter or so from the roof of the nasopharynx. Its other end is attached to a suction pump, and it is convenient to have a hole in its wide part which can be occluded with plasticine. The best shape for the tube must be found by trial, but it is important that it should be out of the way; it may be necessary to carry the palate incision a little farther backwards. The foramen cavernosum of the sphenoid is cautiously located through the mucosa by a dental plastic instrument (fig. 1), and a small pledget of cotton wool is well worked up with a paste of 'Adrenalon' powder (Gedeon Richter Ltd., London). The suction is then turned on, and the mucosa boldly incised down to the bone with a long narrow knife, the incision starting over and being carried forward a few millimeters from the foramen. The resulting rush of blood is carried off by the pump, but as soon as the incision is packed with the prepared cotton wool, bleeding stops altogether. The actual loss of blood is very small. After a few minutes the packing is cautiously removed, with the pump on, and if the hemorrhage recurs it is repacked.

If the foramen has not actually been entered, bleeding stops very quickly, the mucosa appearing blanched. Apart from the incision the mucosa should be uninjured, and the lateral walls should not have been touched. The suction tube is removed.

Injection of Bone Wax. There are two difficulties in this step. First, the wax may solidify in the needle of the syringe when this is removed from the hot water bath, and secondly the body of the syringe may completely obscure the operative field so that the needle cannot be guided into the foramen. These difficulties are overcome by using a syringe of the type employed for syringing out dental cavities with water (fig. 1). The end piece, representing the needle of an ordinary syringe, is so long that the body of the syringe is well out of the way. It is also much wider than the ordinary hollow needle except where it tapers to the point, so that the wax keeps warm and remains fluid. The syringe has the further advantage that its two parts are screwed together. The foramen is nearly always large enough to take the end of the syringe; in exceptional cases it needs enlarging a little with the end of a pair of fine forceps. It has been found best to inject a drop of wax first, give it time to solidify and then follow up with a little more. This seems to occlude some path of least resistance, possibly into an air-sinus, which may otherwise carry off over 1 cc. of wax. Not more than 0.25 cc. is necessary.

If the point of the knife actually enters the foramen when the mucosa is being cut, intractable hemorrhage may occur, and the best way of controlling this is to inject the bone wax before attending to the hemorrhage from the mucosa. There is then danger of a little wax blocking the suction tube. This procedure accounts for the early injection of wax in rabbit *12*, table 1.

Exposure of the Pituitary. The sphenoid being clean, the bone wax injected and hemorrhage stopped, the suction tube is removed and the sphenoid excavated with a burr driven by a dental motor. Perfect visibility is attained by lengthening the shaft of the burr with a piece of steel rod of the same diameter (fig. 1) fixed on with a band of sheet copper and solder. The burr rests in the foramen cavernosum which is enlarged anteriorly and laterally. For about a millimeter anterior to the foramen the bone is thick: after this it is thin. Slight hemorrhage may occur from the mucosa, which tends to overhang laterally, and from the cavity which is exposed. The former may be stopped by 'adrenalon' and the latter by firmly (but gently) pressing small solidified drops of wax into the hole with a blunt probe or a piece of damp cotton wool in forceps. Frequently no hemorrhage occurs at all. When the dimensions of the hole are about 5 mm. long by 3 mm. wide further drilling is usually unnecessary. Visibility is improved by seizing the overhanging bits of mucosa with forceps and cutting them off with a Bowman's needle (fig. 1) or a small knife. A cavity of varying depth is exposed, and by pushing any bone wax to the sides the pituitary capsule can be seen at the bottom with the gland showing white through it. Any further enlargement of the hole which is necessary is now made so as to give as good exposure as possible. It is unwise to carry the excavation too far forward. It is useless to proceed until all oozing has been stopped, as one drop of blood

TABLE 1. TIME, IN MINUTES AFTER BEGINNING OF PALATE INCISION, AT WHICH THE VARIOUS STAGES WERE COMPLETED

Rabbit No.	Palate incision complete	Sphenoid mucosa incised	Bone wax injected	Dental burr (intermittent)	Pituitary completely removed	Palate suture complete	Remarks
1	4	?	28	38–65	—	—	Lateral mucosa damaged. Troublesome hemorrhage. Death at 65 min. from inhaled blood.
2	3	9	20	27–40	57	72	[1]
3	1	12	29	33–45	48	59	Pituitary removed intact.
4	2	5	21	23–38	64	80	Less pituitary removed than expected; much time spent in making sure sella was empty. A piece probably lost in pharynx.
5	2	6	23	28–43	56	74	
6	1	8	26	28–47	—	—	Unable to find gland. Brain entered and rabbit killed at 68 minutes.
7	3	11	32	36–45	77	99	Roof of sella damaged, with escape of c.s.f. Hemorrhage during palate suture.
8	3	8	18	30–33	52	65	
9	1	4	15–17	19–27	40	50	
10	1	5	19	24–?	44	55	Nasal discharge. Fasted for 48 hours.
11	1	8	21	25–64	—	—	No foramen cavernosum. Pituitary not located and brain finally entered. Rabbit killed.
12	2	9	11	23–27	51	68	Deep pituitary.
Average times	2	8	22	28–43	54	69	

[1] All the rabbits which survived the operation made a perfect recovery except 2. This one had a nasal discharge exactly the same as 10. It was given several injections of glucose, and after a 48-hour fast, feeding was attempted by stomach tube. The animal struggled, collapsed, and a few hours later died. It was a vigorous rabbit, and if left alone like 10 would probably have survived.

now suffices to obscure the field. It is at this point that abrasion by swabs or instruments (particularly by the shaft of the burr) of the mucosa on the lateral walls of the nasopharynx becomes a nuisance.

Removal of the Pituitary. This can be accomplished rapidly by slitting the capsule and applying a suction tube to the gland. It is, however, more satisfying to spend a little more time at this stage, which has probably been reached in 30 to 45 minutes. The capsule is very tough and tends when cut to form lateral 'curtains' which hold down and obscure pieces of the gland. It can be removed fairly satisfactorily by running the Bowman's needle round its edge, instead of just cutting into it, the right and left hands being used in turn. This is difficult to do perfectly, but by using forceps, etc., the upper surface of the gland is completely exposed. A small sharp spoon (fig. 1) is now introduced lateral to the gland, and again using each hand in turn, gently worked right round until the gland is free. The stalk is cut during this procedure. The pituitary generally breaks up on being lifted out: only once has it been obtained intact. If it has been freed completely in the first place, there is no difficulty in removing the remaining fragments. The cavity can now be cleaned if necessary by suction, the end of the tube being kept well to the posterior part of the roof of the sella. The walls are scraped with the spoon and the sella thoroughly explored, particularly in the antero-lateral parts which are most difficult to see. Care must be exercised in this region not to penetrate the roof of the sella with the spoon.

The cavity is now filled with solid or molten bone wax, the nasopharynx swabbed with 'Dettol' and the palate sutured.

Survival. At no time has actual inability to remove the gland been a cause of failure, but after numerous apparently successful operations the rabbits died 12 to 36 hours later. Usually no cause of death could be found postmortem, and it was thought that some defect in after-treatment was the cause. Sepsis was never found. Permanent recoveries, however, coincided with the perfecting of the foregoing technic, and it can only be concluded that previous animals died either as a result of too extensive local damage, or from delayed shock. No after-treatment, beyond keeping quiet in a warm room, is necessary. The animals drink freely immediately on coming round and generally start to eat about 24 hours later. One rabbit (10, table 1), which developed a nasal discharge, was none the worse for an apparent fast of 48 hours. A fortnight after the operation this animal's blood sugar fell from 82 to 71 mg. per cent after being deprived of food for only 7 hours, and White's precaution of always maintaining a supply of food in the cage probably becomes more important with the lapse of time.

Miscellaneous. The instruments are kept sterile, but no exaggerated precautions need be taken. The lamp, dental machine (except the burrs) and syringe are not sterile and are handled when necessary. 'Dettol' swabs are used occasionally and nothing dirty is allowed in the wound. An occasional nasal infection supervenes, but clears in a day or two.

Injury to the hypothalamus is to be avoided on principle, and the reason for abandoning suction as a method of removal was the fact that a sudden pull on the stalk might have contributed to the delayed mortality. On three occasions, however, the roof of the sella has been cracked, with escape of cerebrospinal fluid, with no apparent untoward effects (e.g. 7, table 1).

Great anatomical variations are encountered in the width of the nasopharynx, the depth of the pituitary, and the degree of ossification of the roof of the sella. Occasionally a second thin lamina of bone may cover the inferior surface of the pituitary. Rabbits of 2.5 to 3 kg. are suitable in size. In larger rabbits the gland tends to be very deep.

Three glass suction tubes have been used (fig. 1): one with a wide curved end for clearing mucus, etc. from the pharynx, one with a fine straight end for cleaning the sella, and one with a long shaft and a slight bend in the thin part for use during section of the sphenoid mucosa. The last has to be clamped in position during use but the other two are merely slipped into the tubing from the pump when required and held in the hand.

Palate suture is performed with 3/16″ full curved needles held in artery forceps.

RESULTS

Table 1 is a summary of the last 12 consecutive operations. It will be observed that the approach is fairly rapid and most of the time lost is in the last part of the operation. If suction had been employed for removing the pituitary, it is safe to say that the animals would have been off the table at the times given in the sixth column. The last column indicates most of the

difficulties likely to be encountered. A 75 per cent recovery may reasonably be expected. Rabbit 2 in this series was killed by too zealous after-treatment, and may be counted as a successful operation. Failure to find the pituitary, as in 6, is rare, and 11 was unique in having no foramen: the occurrence of these two rabbits so close together is misleading. On the other hand, it should be noted that 7, which might have been expected to die, made an uncomplicated recovery. As the animals were killed, the contents of the pituitary fossa were examined histologically, with negative results. Only a few shreds of the capsule and a little structureless debris, possibly from the bone wax, were left after treatment with xylol.

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SIGNIFICANCE OF OPERATIVE TECHNIC IN THE REMOVAL OF THE PITUITARY

A NEW TRANSPHENOIDAL APPROACH TO THE PITUITARY OF THE DOG AND NOTES ON THE BUCCAL APPROACH TO THE PITUITARY IN THE RABBIT

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ADVANCE in physiological knowledge has often depended upon the technical development of an operative procedure. The contributions of operative technic to biology are comparable to those of analytical-chemical determinations. Owing to its surgical inaccessibility, difficulty has been encountered in removing the pituitary in mammals and the results following hypophysectomy have varied with the operative approach used. It is the purpose of this paper to describe a new operative technic in the dog which has proved advantageous in differentiating between effects of pituitary removal and those of injury to the brain.

From 1886, when Horsley (1) first recorded the successful removal of the pituitary, until as late as 1925 (2) discussion concerning pituitary function centered on whether or not the pituitary is essential for maintenance of life. Present knowledge suggests that the difficulty in answering this question depended upon two things: first, the operative technic used, and second, the failure to understand the intimate relation of the anterior pituitary to carbohydrate metabolism. Aschner (3) in 1912 was the first to appreciate the significance of the operative approach in relation to the survival of the animal. In an excellent detailed account of the function of the pituitary he pointed out that the paralyses, tetany, and convulsions ending in death the first days after hypophysectomy, were probably the result of damage to the brain incurred by the operative manipulations. The factors involved in brain tissue damage have been further analyzed by Dandy and Reichert (2) in 1925.

Although Cushing (4) described an increased glucose tolerance in the absence of the pituitary, and Aschner (3) decreased adrenalin glycosuria, it was not until the work of Houssay that the direct significance of the anterior pituitary to carbohydrate metabolism was understood. Houssay (5) was the first to describe spontaneous hypoglycemia of the hypophysectomized animal. The hypoglycemia follows fasting, and if unrelieved ends in convulsions and death.

A review of detailed protocols available in this field of experiment suggests that many of the deaths 24 hours and more after hypophysectomy are attributable to spontaneous hypoglycemia. Those operations which hampered mastication or deglutition tended to lead to starvation and, therefore, spontaneous hypoglycemia. The temporal approach of Paulesco (6) and Cushing (7), with its damage to the major muscle of mastication, made the postoperative feeding of the animals difficult.

Aschner using the buccal approach through the soft palate with its relatively slight disturbance to eating, was able to maintain the necessary carbohydrate reserves and obtain survival of his animals.

That the pituitary is not essential for the maintenance of life has been settled. There remains unsolved, however, the exact origin of other supposed pituitary functions, in the solution of which the operative technic is of primary importance. Brain damage incidental to hypophysectomy may be in part responsible for adiposity, diabetes insipidus, and carbohydrate metabolism changes. Aschner (3) believed that the adiposity which developed in adult dogs following hypophysectomy was due to brain damage. Bailey and Bremer (8) questioned his conclusions. Smith (9) has clearly shown the significance of hypothalamic damage in the production of obesity in the rat.

The experimental lesion necessary to produce diabetes insipidus varies from one animal species to another. The particular neuro-anatomy of the various animal species, and the presence or absence of anterior lobe, control the appearance of diabetes insipidus. In the solution of such a complicated problem a consideration of operative technic is of fundamental importance.

In the field of carbohydrate metabolism the recent revelations of the function of the anterior lobe have overshadowed the less well understood roles of the posterior lobe and the hypothalamus. In 1935 Chaikoff, Reichert, Larson and Mathes (10) demonstrated permanent changes in carbohydrate balance as a result of the operative manipulations to the brain. In spite of the fact that in the original description of the method (2) the significance of brain damage was realized, Reichert still uses the trans-temporal approach.

HYPOPHYSECTOMY IN THE DOG

Technical Considerations

In the study of endocrine function by ablation of a gland there are two requirements of an operative technic. An unhampered view of the entire organ and the neighboring structures is the first. The second requirement is that in obtaining the necessary view no structures should be interfered with, damage to which might in any way complicate the results obtained from the ablation of the gland. This is true in the removal of the pituitary. A good view can be obtained only by direct vision in a bloodless field. Blind operations by such procedures as suction or the stereotaxic instrument of Horsley will always be of doubtful value. Sufficient anatomical variation occurs in animals of a given species to make the result of such operations unknown until postmortem examination. There is a large element of chance which is unnecessary and uneconomic.

In our opinion there are six major disadvantages of the temporal approach to the pituitary of the dog.

a). The direction of vision is at a poor angle. The surface of the hypothalamic portion of the brain lies parallel to the line of vision and it is only with considerable brain retraction that it is possible to visualize the upper portion of the stalk, the pars tuberalis of the glandular tissue, and the surface of the brain surrounding the infundibular stalk. It is, therefore, impossible to visualize clearly the entire pituitary field even though a good view of the main lobes of the gland can be obtained. Because of this difficulty and in order to insure complete removal of the stalk and of the glandular tissue, cauterization of the base of the brain has been used by many workers. It is

impossible to control accurately the extent of nerve cell damage and edema from cauterization.

b). Brain tissue damage is inevitable. In an attempt to increase the visual field there is an unavoidable retraction of both the temporal lobe and the hypothalamic regions. No matter how low the trephine opening into the temporal fossa is made (11) it is necessary to place pressure upon the brain in order to lift the pituitary out of the shallow sella turcica. Dandy and Reichert (2) advised rotating the dog over on the back so that the brain fell away from the floor of the skull. This diminishes pressure upon the brain but according to their own experiments does not completely obviate lasting brain injury. In our own hands no matter how carefully the brain has been retracted we have noticed transient postoperative weakness of muscles on the opposite side of the body, suggesting damage to the motor cortex.

c). The position of the third nerve complicates the freeing of the attachment of the posterior lobe to the posterior clinoid process.

d). The vein which drains the hypothalamic brain and empties into the cavernous sinus lateral to the pituitary stalk, prevents an uninterrupted view of the stalk. Retraction or division of this vein in order to obtain a better view courts severe hemorrhage.

e). Incision, division or dislocation of the temporal muscle necessary to visualize the pituitary by the temporal bone approach causes difficulty in eating after operation. This handicaps the surmounting of the nutritional aspect of pituitary insufficiency.

f). With re-suture of the temporal muscle over the operative bone defect the dura becomes adherent directly to muscle and mastication results in brain massage.

The only advantage of the temporal approach to the pituitary is that it enables complete aseptic surgical technic. It is possible to exclude infectious meningitis as a postoperative complication.

Approach to the pituitary of the dog through the floor of the sella turcica obviates the disadvantages of the trans-temporal route.

There is, however, a definite disadvantage to the transphenoidal approach. The field of vision although at a correct angle to the brain is limited in circumference. The cavernous sinus rings the sella turcica. Attempts to retract the cavernous sinus usually result in profuse hemorrhage. By the buccal transphenoidal approach because of the anterior connecting sinus between the lateral cavernous sinuses, it is difficult to obtain a clear view of the base of the pituitary stalk. In the parapharyngeal transphenoidal method described in this paper this difficulty is overcome by approaching the pituitary from an angle farther posteriorly. This new angle of approach introduces a difficulty due to the posterior connecting vein not ordinarily seen by the buccal approach. This posterior vein often makes it difficult, sometimes impossible, to see the posterior clinoid process but it is preferable to visualize clearly the base of the brain rather than the posterior clinoid process. The posterior lobe pulls away easily and completely from its vascular attachment. A limitation of the circumference of vision is less important than the direction of vision. The difficulties of the former but not of the latter can be overcome with experience.

It is technically difficult to expose the base of the sphenoid aseptically. By the buccal approach, organisms both from the mouth and nasopharynx

are introduced into the operative field. The parapharyngeal operation can be done aseptically. We have not, however, hesitated to open into the nasopharynx as will be described as an alternate procedure in the account of the operation. By giving up hypophysectomy in the winter months all but minor sepsis in the nasopharynx has been avoided.

Description of Parapharyngeal Transphenoidal Approach

Ether has been administered through a large bore, thick walled rubber tube inserted through the mouth into the trachea. The dog is turned on its back, the head placed on a pad to elevate and steady it. The lower jaw is secured firmly by rope or wire to the table (fig. 1). Routine aseptic skin preparation with iodine solution is made. The skin incision is made as shown in figure 2. Made on either side, it runs from the hair mole anteriorly to the upper border of the larynx posteriorly. The lateral aspect of the constrictor muscles of the pharynx are exposed by sharp dissection (fig. 3). The hamulus of the pterygoid process is exposed by separating the pharyngeal constrictors from the temporal muscle. The smaller vessels are retracted laterally, the dissection being readily accomplished and almost bloodless. The superior constrictor muscles are next freed from their attachments to the hamulus and the dissection carried to the base of the sphenoid. Care must be taken not to open into the pharynx. Exposure to the midline is obtained with a large self-retaining retractor which carries the intratracheal tube and the pharynx to the far side of the head (fig. 4). The rubber tube permits free airway without tracheotomy.

There is a choice of procedure at the next step. In order to obtain complete asepsis after division of the muscular attachments to the hamulus, the mucous membrane of the nasopharynx is carefully separated from the medial surface of the pterygoid process to the base of the sphenoid bone. The periosteum of the sphenoid bone is incised for 2 cm. along the pterygoid process and freed from the bone across to the base of the pterygoid process on the other side. It is at this point that it is very difficult not to open the mucous membrane since it is closely adherent to the periosteum. It may be necessary to dissect a large area anteriorly and posteriorly in order to obtain a large enough operative field. A small self-retaining retractor can now be used to retract the mucous membrane and periosteum.

The alternate procedure, which accepts bacterial contamination from the nasopharynx, is to open directly into the nasopharynx. The incision is made posteriorly from the hamulus (fig. 4). The mucous in the nasopharynx is sucked out by a glass suction tube and the nasopharynx is shut off with gauze plugs anteriorly and posteriorly. Iodinization of the nasopharynx can also be done if desired. The same small self-retaining retractor is inserted and a crucial incision is made in the mucous membrane and periosteum overlying the body of the sphenoid bone. The four corners of periosteum are freed by blunt dissection (fig. 5). A plate of the outer table of the sphenoid bone, measuring about 12×8 mm., is removed with an electric dental drill.[1]

[1] R. J. Bennett, Jr., (*Endocrinology* 20: 860. 1936) has described an ingenious metal guide and drill for use in the buccal transphenoidal approach to the dog pituitary. We believe these instruments are of limited value since the hemorrhage which they entail results in a blind suction procedure.

The landmarks for the bone excision are as follows. The inferior surface of the body of the sphenoid bone is flat. The junction of the sphenoid and occipital bones is marked by two mammillary elevations with a cleft between, rising from the anterior margin of the occipital bone (fig. 5). On the flat portion of the sphenoid bone there are three landmarks. Just medial to the medial pterygoid process and opposite the tip of the hamulus on either side

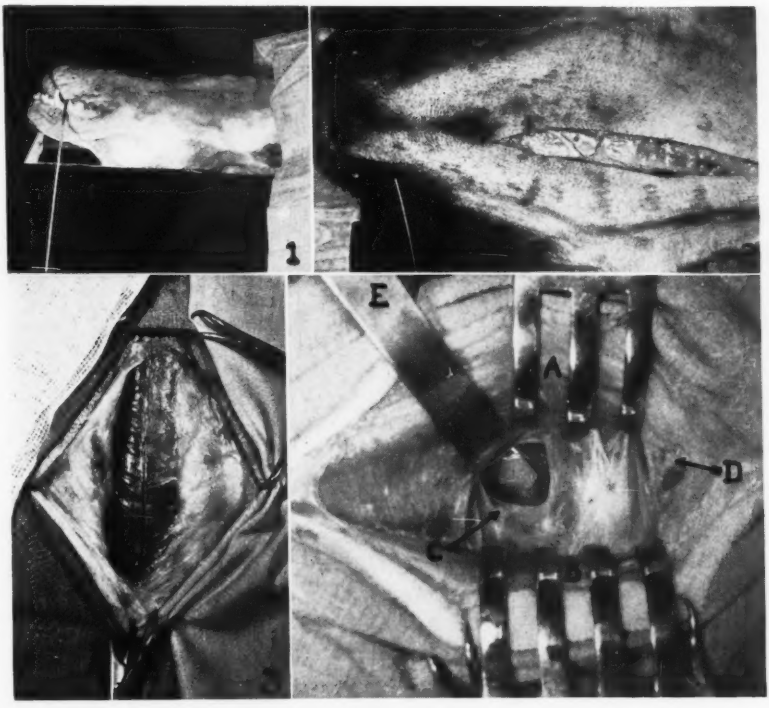


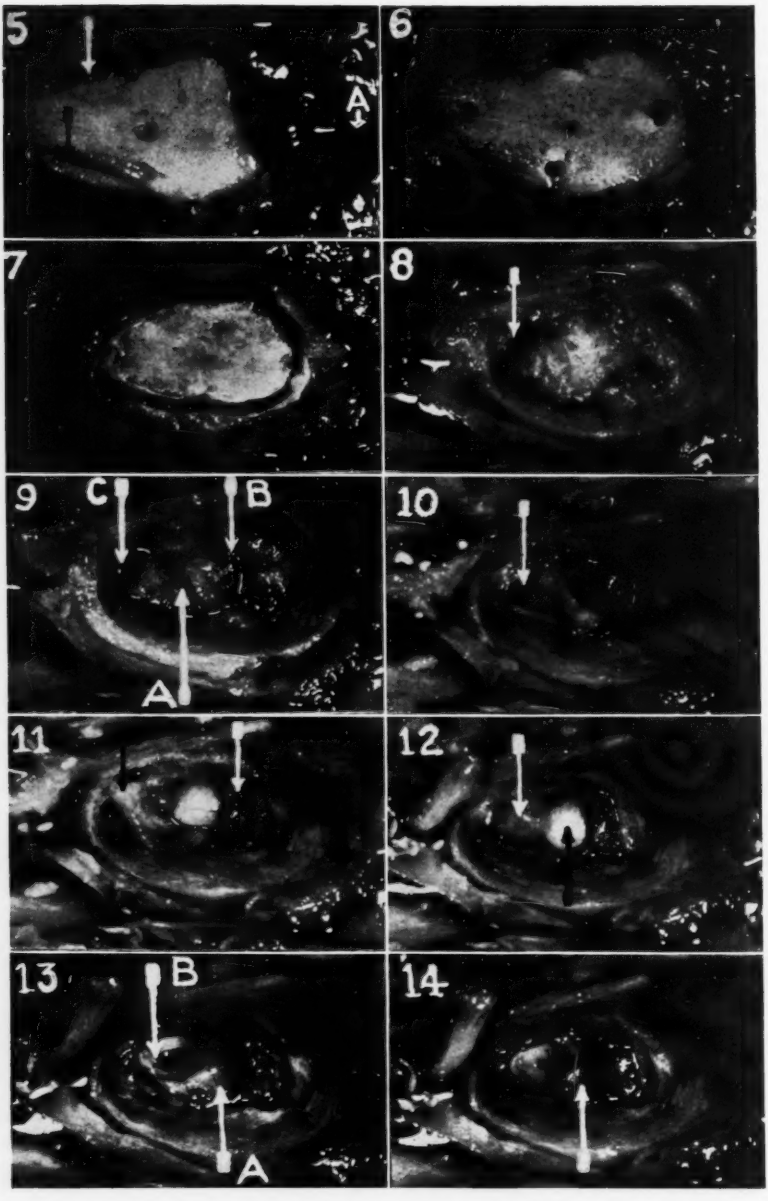
Fig. 1. Dog in position for hypophysectomy. Head fixed to table by lower jaw.

Fig. 2. Site of incision, from hair mole anteriorly to upper border of larynx to left of midline. Intratracheal tube in place. In all subsequent figures, except figure 3, the position of the dog's head remains identical with fig. 2. The left side of photograph is anterior, right side posterior. Lower portion of photograph is, therefore, left side of dog.

Fig. 3. Exposure of pharyngeal muscles. Approach follows the lateral wall of these muscles.

Fig. 4. Sphenoid bone exposed. *A*, pharyngeal muscles. *B*, temporal muscle. *C*, hamulus of pterygoid process. *D*, hyoid bone. *E*, retractor retracting nasopharyngeal mucous membrane exposing the sphenoid bone. The mucous membrane of the roof of the nasopharynx and periosteum of the sphenoid bone have been incised and retracted.

lie two fine slit-like sinuses, about 6 to 8 mm. long. In the center of the body, opposite the posterior ends of these slit sinuses, is a small sinus carrying a vein. When the periosteum is retracted this small vein bleeds, making its identification possible. This central sinus is in the middle of the bone plate to be removed. The posterior end of the plate is the posterior margin of the sphenoid bone. The lateral boundaries of the plate are the slit sinuses.



Figs. 5–14 (*See opposite page for explanation*)

There is no definite landmark to determine the anterior end of the plate (fig. 5, 6, and 7). Bleeding is readily stopped with bone wax.

When the outer bone plate is removed, medullary bone is curetted out, uncovering the floor of the sella turcica (fig. 8). In order to get an adequate view anteriorly the cancellous bone of the entire anterior clinoid process is taken away. The inner table of the sphenoid bone or actual floor of the sella turcica is then attacked with a burr drill, exposing the dura without opening it (fig. 9). Laterally, the inner table is drilled away until the cavernous sinus is clearly visible as a blue line through the dura. Anteriorly, the dissection is limited by the anterior communicating vein which lies in the dura just anterior to the anterior clinoid process. The entire bony process can, therefore, be removed.

In the excision of the inner table posteriorly the posterior communicating vein is exposed before the posterior clinoid process is reached (fig. 9). At this point we have found it wise to stop the bony excision since the posterior communicating vein has afforded the greatest operative difficulty. We have tried dividing it after cauterization or occlusion with silver clips. Neither method has been sufficiently successful to warrant its continued use.

The dura is next opened with a T-shape incision, the top of the T lying parallel and just anterior to the posterior communicating vein. The major pituitary lobes are now pushed by the spinal fluid pressure directly into the dural opening. When the gland is depressed 2 to 3 cc. of spinal fluid well up, relieving the intracranial pressure; the brain drops away from the wound and the pituitary slips back into the cranial cavity. The pituitary is gripped with a glass suction pipette and pulled posteriorly, exposing the brain and pituitary stalk.

Fig. 5. Base of sphenoid bone, nasal mucous membrane and periosteum reflected. Unmarked arrows point to lateral venous sinuses. Arrow A points to cleft between mamillary elevations of occipital bone. In middle of exposed bone is small drop of blood which has oozed from the small central venous sinus.

Fig. 6. Exposed base of sphenoid bone with preliminary drill holes showing use of lateral venous sinuses as landmarks.

Fig. 7. Outer or inferior table of sphenoid bone isolated with drill.

Fig. 8. Inner table of sphenoid bone or floor of sella turcica. The outer table and cancellous bone have been removed. The arrow points to the excavated anterior clinoid process.

Fig. 9. Dura exposed. Inner table of sphenoid removed. A, anterior pituitary seen through dura. *B*, posterior communicating vein running between lateral cavernous sinuses. C, excavated anterior clinoid process.

Fig. 10. Dura incised and retracted. Anterior lobe retracted posteriorly and to the right with dissector revealing its attachment along stalk. Arrow points to stalk.

Fig. 11. Posterior lobe and posterior half of stalk exposed by dissecting right half of anterior pituitary free. Black arrow marks retracted right lobe of anterior pituitary. Its unsevered connections to the stalk are visible. Left anterior lobe still in situ. White arrow marks posterior communicating vein. Dural flap seen retracted along sphenoid bone. Large vessel running to posterior clinoid process is seen on inferior surface of posterior lobe.

Fig. 12. Anterior lobe resected. Black arrow points to posterior lobe. White arrow points to pituitary stalk. Seen in this position the pituitary stalk is short and bulbous. By the temporal approach the weight of the pituitary lobes stretches out the stalk so that it is elongated and thin.

Fig. 13. Posterior lobe removed with a portion of infundibular stalk. Arrow *B*, remaining portion of stalk. Arrow A, left corpus mamillare of brain.

Fig. 14. Entire pituitary lobes and remnant of stalk removed. In the center of the tuber cinereum, from which the stalk has been dissected, the prolongation of the third ventricle into the pituitary stalk can be seen as a jagged edge. Arrow points to left corpus mamillare.

In fig. 5 to 14 the position of the dog's head is the same as in fig. 2. Magnification, fig. 5 to 14, ×2.

The glandular tissue and the neural stalk can be readily differentiated from the brain (fig. 10 to 14). The ordinary head lamp is used for illumination. The dissecting microscope has not been found necessary but may be fitted into the operative field if desired.

Pituitary removal is best started by freeing the stalk with sharp dissection from the base of the brain. Only transient bleeding occurs from the small vessels which quickly retract. When the stalk has been removed the infundibular recess of the third ventricle is clearly visible (fig. 14). All of the attachments of the anterior lobe in the dog are to the infundibular stalk (fig. 10, 11). The anterior lobe comes away when the stalk and posterior lobe are freed. Either by teasing with a blunt dissector or by grasping both lobes in a pair of forceps, the posterior lobe is pulled free from its vascular attachment to the posterior clinoid process. Following this procedure there is sometimes brisk hemorrhage which may be controlled by brief pressure with a pack against the posterior clinoid process.

It is important to appreciate that in the dog separate removal of either the anterior or posterior lobe of the pituitary, leaving the other lobe functionally intact, is probably impossible. Although in the accompanying illustrations the anterior lobe tissue was removed revealing the stalk and posterior lobe in situ, either small rests of anterior lobe cells are left along the stalk or the stalk with its nerve pathways is damaged in removing all anterior tissue. The anterior lobe of the dog receives its entire blood supply along the stalk and sometimes in removing the anterior lobe the stalk is pulled out of the posterior lobe before these vascular connections give way.

Removal of the posterior lobe and infundibular stalk necessitates destruction through its blood supply of the entire anterior lobe. The posterior one-half to two-thirds of the posterior lobe may be left intact with its vascular attachments from the posterior clinoid process, all of the anterior lobe tissue having been removed. The only posterior lobe hormone remaining to an animal so operated would be that secreted into the blood stream in the absence of nervous stimulation.

In closing the wound a small piece of muscle is excised from the constrictor muscle of the pharynx and placed in the dural defect in the floor of the sella turcica. If no blood can be obtained by scraping the sides of the sphenoid bone, 1 cc. of blood is removed by needle or incision from one of the veins in the superficial part of the incision and placed in the bony defect and watched until clotted. It is imperative that a firm blood and muscle clot be left in this bony defect before the operative wound is closed. In this way loss of spinal fluid and ascending infection from the naso-pharynx into the cranial cavity are prevented. Stitches can be taken in the periosteum of the sphenoid to help hold the clot in place but are not necessary. Incision in the nasal mucous membrane is closed with a fine cat-gut suture. A few single silk sutures are taken in the subcutaneous fascia, and the skin closed without drainage.

The wounds have healed promptly. With minimal dissection of the pharyngeal constrictor muscles no difficulty in swallowing occurs after operation.

Results of experiments on dogs operated on by this method are published elsewhere (12).

HYPOPHYSECTOMY IN THE RABBIT

What has already been said concerning the temporal approach to the pituitary in the dog applies in reality to all mammals. In those animals whose main pituitary lobes lie in a deep or encasing sella turcica, the difficulty of removing the pituitary via the temporal approach is obviously increased. Cauterization with chemical substances such as chromic acid was used in some of the first recorded experiments and resulted in uncertain hypophysectomy and some degree of chemical damage to the brain tissue.

Firor (13) has suggested an ingenious approach to the pituitary of the rabbit through the back of the orbit. This is a blind technic since the sella turcica is not visualized. The pituitary is removed by a curved suction cannula. Although it is possible to feel clearly the confines of the sella turcica with the cannula it is not possible to remove that portion of the glandular lobe, the pars tuberalis, lying above the roof of the sella. Attempts to reach the upper portions of the stalk through the foramen in the roof of the sella turcica would lead to an unknown amount of brain damage. Where a rapid method for subtotal hypophysectomy is required in the rabbit this method is excellent since the mortality rate is low.

The first successful total hypophysectomies in rabbits were reported by Smith and White (14). Only a brief outline of the technic was given. Later White (15) gave a detailed description of the operative procedure.

In first performing hypophysectomy in rabbits we followed White in detail. With increasing experience ways were found for obviating some of the difficulties. These experiences we believe are worth recording in detail. The results of the experiments have been recorded elsewhere (16). In following the operative details reference should be made to White's illustrations. We have used the excellent rabbit board and head holder designed by Smith and White. The tongue retractor has been altered to insure a clear airway. A simpler and less traumatizing soft palate retractor has been devised.

The greatest operative difficulty in the rabbit is contending with the nasopharynx. The rabbit breathes only through the nose and the commonest cause of death after hypophysectomy is nasal obstruction. Mechanical or thermal trauma to the mucous membrane and submucosa may result in sufficient hemorrhage or edema to block completely the nasal passage with death in the first days after operation. In winter months the postoperative edema has been so increased, apparently owing to the prevalence of upper respiratory infections, that we have abandoned hypophysectomy from fall to spring. The parapharyngeal or aseptic operation in the rabbit is impossible, not so much because of the narrowness of the nasopharynx as suggested by White, but because of the vascularity of the submucosa. The measures necessary for the control of hemorrhage cause fatal postoperative nasal obstruction.

The complication of loss of spinal fluid, stressed by White, can easily be avoided. Infecting organisms from the pharynx alone remain as unavoidable. We have, however, had but few if any fatalities directly ascribable to meningitis.

Operative Procedure

From the foregoing discussion it is obvious that in the operative procedures the greatest care must be taken to avoid traumatizing the mucous membrane and submucosa of the nasopharynx. In dividing the soft palate a hook-point scalpel has been found convenient. To inject the palate with adrenalin solution is unnecessary and only adds to the difficulty of exposure. If the incision is made accurately in the median raphé no bleeding will occur unless the incision is carried too far anteriorly toward the hard palate where a transverse artery lies. Retraction of the soft palate is accomplished by a light spring, short pronged wire loop retractor. Before exposing the sphenoid bone or roof of the nasopharynx it is wise to obliterate the rich vascular network lying between periosteum and mucosa. White advised prolonged pressure with gauze to accomplish occlusion, a procedure requiring some 20 minutes. The vessels in the operative field converge in a rich venous sinus to the cavernous foramen, passing through the sphenoid bone into the cavernous sinus. This connecting sinus has to be obliterated later before the bone is drilled and both procedures may be accomplished at one time. Smith and White originally recommended injection of hot wax in the cavernous foramen but White later abandoned it. It is, however, a most useful procedure. With a short bevelled 22 ga., 1-inch needle on a long barrelled 1 cc. syringe, 0.2 to 0.3 cc. of hot wax is injected directly. The wax and syringe must be as hot as can be handled. The cavernous foramen is identified as soon as the nasopharynx is exposed with a pair of blunt ended forceps. The needle is inserted 2 mm. beneath the mucous membrane and the wax injected just rapidly enough to keep it from solidifying in the needle. Half of the wax is run into the sphenoid bone and half is allowed to run out into the submucosa outside the sphenoid bone. The needle is held in place until the wax solidifies. Incision can then be made anteriorly from the cavernous foramen and the mucous membrane retracted laterally without any bleeding. Care must be taken not to insert the needle too far nor to inject too much wax because the wax will find its way past the cavernous sinus and rupture through beneath the dura into the cranial cavity.

The advantage of this technic is that the operative time of 45 minutes to 1 hour required by White is cut to 15 to 20 minutes. Although the mechanical trauma to the nasal cavity is decreased there is danger of burn from the hot wax.

The excision of the sphenoid bone is carried out as recommended by White. The inner table of bone is removed usually with a blunt dissector. In order to avoid the cavernous sinus care must be taken not to carry the bone removal too far laterally. The entire bony anterior clinoid process is removed.

There are two major differences in the anatomy of the pituitary of the dog and the rabbit. In the first place the anterior pituitary in the dog receives its entire blood supply from the neural stalk while in the rabbit there is a vascular network attaching the anterior lobe to the dura of the anterior clinoid process. It is, therefore, possible in the rabbit to leave portions of

anterior lobe with intact blood supply and at the same time remove the entire neural hypophyseal apparatus. Secondly, the major pituitary lobes in the dog lie free in the cranial cavity. In the adult rabbit the anterior and posterior lobes are walled off from the hypothalamus by a cartilaginous roof of the sella turcica. This roof develops only as the rabbit reaches maturity. In the baby rabbit only a fold of dura is present posteriorly. In older rabbits the cartilage may become calcified. In the mature rabbit with developed roof the neural stalk passes through a foramen which may measure not more than 1.5 mm. in diameter. A small portion of the glandular tissue or pars tuberalis lies above the foramen in the cranial cavity. When this foramen is small it is obviously necessary to enlarge it in order to remove the entire glandular tissue as well as the neural stalk.

The dura is opened first anteriorly around the stalk. Transient bleeding occurs from the vascular connections of the anterior lobe after which a clear view of the stalk is obtained. The stalk is then freed from the brain by the sharp ended dissector, retracting the stalk by suction tube. It is essential to disconnect the stalk from the brain first since if the pituitary is removed first, as recommended by White, a portion of the anterior lobe in addition to the pars tuberalis may be drawn into the cranial cavity attached to the stalk. It is also easiest to see the base of the brain when the dura is first opened. With the continued escape of spinal fluid the base of the brain falls away from the sella turcica pulling the stalk with it. Later on in the operation it may be difficult to recover the pituitary stalk.

The contents of the sella turcica with stalk attached are next removed. This is best accomplished by removing the entire dural lining as well as the lobes contained therein. Because of the narrowness of the nasopharynx it is impossible even with the dissecting microscope to visualize the lateral walls of the sella turcica. White found that in some of his operations pieces of anterior lobe had been left along the lateral walls of the sella. If the entire dural lining is stripped out, a maneuver easily accomplished by blunt dissection, there can be no doubt about the removal of all glandular tissue and postmortem serial sections of the sella turcica itself are not needed. The only area requiring microscopic observation postmortem is the brain. This is done in order to insure complete removal of the pars tuberalis as well as the neural stalk.

There is occasionally some hemorrhage from the vascular attachment of the posterior lobe to the posterior clinoid process. This is rapidly relieved by pressure pack. The sella turcica is allowed to fill with blood clot to which a small piece of muscle from the soft palate is added. As in the dog it is important that this clot should be firm in order to prevent loss of spinal fluid and to prevent ascending infection. Further use of bone wax is contraindicated since the more unabsorbable foreign body added the greater the inflammation. No attempt is made to close the mucous membrane of the roof of the nasopharynx but the soft palate should be accurately approximated with one or two fine stitches. We have lost 2 animals some weeks after hypophysectomy from blockage of the nasal passages with finely chewed

cabbage which had found its way up through a persistent hole in the soft palate.

The postoperative nutritional care of the animals in order to avoid hypoglycemia is of course important. The appetite of the hypophysectomized animal is finicky; it will eat sometimes only the freshest foods.

SUMMARY

Historical survey of the removal of the pituitary in experimental animals suggests that the early failures to obtain survival were due in part to the operative technic employed. There are still problems of supposed pituitary function wherein considerations of the operative technic are significant. The advantages of a new parapharyngeal approach to the pituitary in the dog are enumerated. The operative technic is given in detail. A description of the modified technic of the parapharyngeal approach in the rabbit is appended.

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ADDENDUM

There is one essential difference in the technic employed by Dr. W. H. Newton and described in the preceding article (pages 468 to 474), from that used in our laboratory. Dr. Newton removes only the contents of the sella turcica. He intentionally avoids opening into the general cranial cavity. The fact that spinal fluid only occasionally flows into the operative field means that enough of the pituitary stalk is left to block the foramen through which the stalk passes from the general cranial cavity to the sella turcica. In our own operation we intentionally remove the entire pituitary or infundibular stalk to as far as the base of the brain. We accept the necessity of opening into the general cranial cavity and do this anterior to the pituitary stalk before we remove the contents of the sella turcica.

Serial sections of the base of the brain, infundibular stalk and both pituitary lobes, show that the cells of the anterior lobe extend upwards around the posterior pituitary stalk into the general cranial cavity. Indeed there may be a collection of cells above the fold of dura which forms the foramen around the stalk. These cells correspond to the pars tuberalis encountered in other animals. The number of unoperated animals examined by serial section in our series is too small to state whether these anterior lobe cells are a constant finding in all species of rabbits but we believe there is sufficient evidence to assume their existence unless proven absent. For this reason also serial sections of the contents of the sella turcica post mortem are not sufficient to determine presence or absence of residual pituitary tissue. It is necessary to examine by serial section the brain lying at the base of the infundibular stalk.

There is a second reason why we believe the general cranial cavity must be opened into. The large proportion of the blood supply of the anterior lobe is received through vessels lying in the fold of the dura forming the anterior margin of the foramen. Unless this portion of the dura anterior to the foramen

is removed it may be difficult to remove all the cells of the anterior lobe which may cling to the vessels. The proportion of anterior lobe cells which may remain along the pituitary stalk compared with the total number of anterior lobe cells is of course small. An animal operated upon by Newton's method would behave essentially, in its early days at least, like an animal in whom all of the cells had been removed. The danger of leaving even a few pituitary cells comes when the experimental animal is kept for 2 weeks or longer, when considerable regeneration of anterior lobe tissue may occur. In some of our earlier animals we have reason to believe that this occurred. This regenerated remnant may of course modify experimental results.

It is unfortunate that it is necessary to enter the general cranial cavity and to remove the pituitary stalk since the operation is not only more difficult but the operative mortality higher. It is also a painstaking procedure to remove the stalk and at the same time avoid damaging the base of the brain.

The other differences between Dr. Newton's and our technic are minor and represent merely manipulative preferences. Although he does not describe it as a cause of death, we both agree apparently on the importance of avoiding damage to the mucous membrane of the nasopharynx.

Since this paper was prepared Dr. I. Kapnick, working in this laboratory, has divided the operative procedure into two stages. At the first stage the sella turcica is exposed but the dura and pituitary are not disturbed. At the second stage the pituitary is removed. This should take place after all signs of inflammation of the nasopharynx have disappeared and before too much bone regeneration has occurred. The optimum time between the stages has been found to be between 1 and 2 weeks. In the first stage the major sources of bleeding are eliminated. If there is edema of the nasopharynx the animal with pituitary intact survives the respiratory obstruction without difficulty. At the second stage the old incision in the palate and nasopharynx are re-opened rapidly and without hemorrhage; consequently the operative time of removal of the pituitary is halved. The total time (both stages) varies between 15 and 30 minutes. The advantage of the two-stage technic, however, is not so much that the operative time is shortened but that the mortality due to respiratory obstruction is almost eliminated.

AUGMENTATION OF PITUITARY GONADOTROPIC HORMONE BY CHLOROPHYLL, PLANT GROWTH HORMONES[1] AND HEMIN[2]

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THE MARKED INCREASE in the effectiveness of pituitary gonadotropic extract by addition of heme or hemin which was reported by McShan and Meyer (1) and Casida et al. (2) suggested that chlorophyll, which has a basic nucleus chemically similar to hemin, might also have similar augmentation effect. Preliminary results verified this possibility, and additional experiments were made in which two of the so-called plant growth 'hormones' were also tested for augmentation effect.

MATERIAL AND METHODS

Immature female rats and baby cockerels of the single comb White Leghorn variety were used as experimental animals. Daily injections were made in the rats from the 23rd to the 27th day, and the animals were killed 24 hours after the last injection. The chick series were divided into 3 age groups: in the first, chicks were injected from the 5th to the 14th day after hatching; in the second, from the 5th to the 9th day; and in the third, a single injection was made when the chicks were one day old. Animals were killed in the first two experimental series 24 hours after the last injection, but chicks in the third series were not killed until after a lapse of 72 hours, during which time they were without food or water. This latter method has been described by Byerly and Burrows (3) and is very successful as a critical test for small amounts of gonadotropic hormone. All of the preparations were administered subcutaneously.

Acetone-desiccated whole-hog pituitary powder was extracted with .05N NaOH. The 33% acetone-soluble fraction of this extract was then precipitated with excess acetone and the precipitate redissolved in water. Dosages were calculated on the basis of gram equivalent amounts of the original pituitary powder. Two commercial preparations of chlorophyll were employed, one of which was an alcohol and the other a water-soluble chlorophyll extract. The two plant growth hormones used were indolebutyric acid (auxilin) and *l*-napthalene acetic acid; these also were commercial preparations.

RESULTS AND DISCUSSION

Daily injection of 40 mg. of pituitary extract was sufficient to produce a slight increase in average ovarian weight of the rats and to modify weight

[1] The author wishes to thank the Pennsylvania Chemical Corporation for the plant growth hormones used in this study. Technical assistance was given by the N. Y. A.
[2] Contribution No. 82 from the Waterman Institute and No. 277 from the Department of Zoology, Indiana University.

range (series A). Each of the plant growth substances, when combined with the pituitary extract (series *B* and *C*), produced definite size increase in the ovarian weight averages and these exceeded the average of the group given AP alone. The maximum ovarian weights observed in these series, 44.3 mg., occurred in the *l*-napthalene acetic acid treated rats (series *C*). The difference in average weights between the two series using plant growth chemicals as augmentors, however, does not appear to be significant. The daily administration of 0.05 cc. of the water-soluble chlorophyll after admixture in vitro with the pituitary hormone (series *D*) produced ovaries averaging 19.6 mg., but the addition of 0.08 cc. of the chlorophyll daily (series *E*) resulted in a greater response. The average in the latter series was 27.1 mg. with a maximum ovarian size of 55.4 mg. This difference seems significant and indicates that further study of dosage levels will perhaps be profitable. Alcoholic chlorophyll and pituitary hormone injected together elicited the most marked ovarian responses. Those rats which were given 40 mg. of the pituitary extract with the alcoholic chlorophyll (series *G*) had average ovarian weights of 32.7 mg. with a maximum of 70.0 mg. This is considerably in excess of the response produced either by combinations of pituitary with the plant growth hormones or water-soluble chlorophyll. Augmentation in the chlorophyll series also varied with the amounts of pituitary extract which was administered. Thus 20 mg. of pituitary extract and 0.05 cc. of the alcoholic chlorophyll (series *F*) developed ovaries with a mean of 15.2 mg.; and the same amount of chlorophyll, when combined with 60 mg. of the hormone (series *H*), produced a 50.7 mg. average. Since it was noted previously that the injection of 40 mg. of extract produced a 32.7 mg. average, it can be seen that the increase was proportional to the amount of pituitary hormone used in these experiments. These results with chlorophyll correspond to those reported with heme by Casida, et al.

McShan and Meyer added 0.5% sodium carbonate to hemin to insure its solution, but it seemed that less variation in pH might be encountered if if pyridine were used to dissolve the hemin, since both the pituitary hormone and hemin are soluble in pyridine. When a few drops of pyridine were added the resultant mixture was clear and almost blood red in color, and while this solution was definitely colloidal, it showed no tendency to precipitate. The ovaries of the rats treated with this preparation averaged 19.9 mg. (series *I*), whereas the same amount of hemin and pituitary extract without the addition of pyridine produced mean ovarian size of only 12.0 mg. (series *J*). It is notable that this was less than the increase which resulted when the pituitary hormone was given alone and was not significantly higher than the average for the normal rats, nor did the range of variation suggest stimulation. A larger amount of pituitary hormone, 60 mg., combined with hemin and with a smaller amount of the pyridine added (series *K*), produced ovaries which averaged 17.2 mg. The amount of pyridine used in this instance was not sufficient to dissolve the hemin which remained finely divided and the mixture did not have the typical red color. It is evident, therefore, that the pyridine had no direct effect in the augmentation.

The response of the rats to mixtures of hemin and pituitary are interesting when compared with the results which followed injection of the plant hormones and chlorophyll separately from the pituitary extract. Administration of pituitary hormone on one side of the animal and auxilin on the other was accompanied by average ovarian weights of 15.7 mg. (series *L*). In contrast, the series in which water-soluble chlorophyll and pituitary hormone were injected separately had a weight average of 11.4 mg. (series *M*). The effectiveness of the pituitary hormone, in the second experiment, was apparently inhibited, and this result is comparable to that in the series in which the hemin was incompletely dissolved. It may be noted also that when McShan and Meyer gave hemin and pituitary extract separately the ovarian

TABLE 1. EFFECT OF GONADOTROPIC EXTRACT AND AUGMENTOR ON RATS

Daily treatment	Series	Animals	Ovarian weight in mg. Range	Mean and σ_m
Control		10	7.1 to 14.4	11.2±0.66
40 mg. AP	*A*	15	9.1 to 18.6	13.7±0.73
40 mg. AP+0.1 cc. Auxilin	*B*	13	12.3 to 29.2	20.7±1.4
40 mg. AP+0.1 cc. *l*-Naphthalene acetic acid	*C*	10	14.6 to 44.3	22.7±3.4
40 mg. AP+0.05 cc. H_2O Chlorophyll	*D*	8	10.3 to 30.1	19.6±2.3
40 mg. AP+0.08 cc. H_2O Chlorophyll	*E*	5	10.7 to 55.4	27.1±4.9
20 mg. AP+0.05 cc. Alc. Chlorophyll	*F*	8	10.6 to 25.8	15.2±0.55
40 mg. AP+0.05 cc. Alc. Chlorophyll	*G*	9	12.3 to 70.0	32.7±5.6
60 mg. AP+0.05 cc. Alc. Chlorophyll	*H*	8	14.1 to 69.3	50.7±6.3
40 mg. AP+0.25 mg. Hemin+Pyridine	*I*	14	10.0 to 37.5	19.9±2.0
40 mg. AP+0.25 mg. Hemin	*J*	10	6.2 to 13.8	12.0±0.69
60 mg. AP+0.25 mg. Hemin+Pyridine (not sufficient to dissolve hemin)	*K*	9	11.6 to 25.2	17.2±1.4
40 mg. AP+0.1 cc. Auxilin separately	*L*	8	10.4 to 21.9	15.7±1.3
40 mg. AP+0.05 cc. H_2O Chlorophyll separately	*M*	10	9.7 to 14.8	11.4±0.53

response was less than that produced by an equal amount of gonadotropic preparation given alone. Since both average ovarian weight and range in the auxilin series differed from those in the AP series, the increase seems to be significant. This point also indicates, as in the chlorophyll series, that the mechanism involved must be studied in much greater detail.

Casida, et al. (2) failed to get augmentation in the chick with combinations of heme and pituitary extract. This is puzzling, in view of the responsiveness of the bird to the injection of gonadotropic substances. A similar result, however, was previously noted by Breneman (4) inasmuch as combinations of FSH and LH failed to produce augmentation in the chick. The series in table 2 are of interest because they indicate the presence of factors which were not considered in previous experiments.

The results in 10-day and 15-day experiments (series *N*–*T*) are in harmony with the findings of Casida, et al., inasmuch as there is no significant difference in the testicular weights between the various series. The gonads of the chicks in the 10 and 15-day series which received auxilin and pituitary

extract (series $\mathcal{Q}$ and $\mathcal{T}$) were heavier than those which received the pituitary hormone alone (series $\mathcal{N}$ and $\mathcal{S}$). The significance of these results must be discounted somewhat because of the small number of chicks represented, but an additional bit of evidence of increased response is also added by a study of comb growth.

Comb factors are not included in table 2 because they were of doubtful significance in the 10-day series and too small to measure accurately in the 72-hour experiments. There was, however marked dissimilarity in comb sizes in the 15-day series. Those chicks which received pituitary hormone

TABLE 2. EFFECT OF GONADOTROPIC EXTRACT AND AUGMENTOR ON CHICKS

Daily treatment	Series	Animals	Testis Weight in mg. Range	Mean and σ_m
	10-day series			
Control		27	9.2 to 29.5	17.4±1.3
40 mg. AP	$\mathcal{N}$	10	15.2 to 60.4	36.1±3.0
40 mg. AP+0.25 mg. Hemin	$\mathcal{O}$	10	21.3 to 44.9	32.3±2.1
40 mg. AP+0.25 mg. Hemin+Pyridine	$\mathcal{P}$	9	24.9 to 51.6	35.7±2.9
40 mg. AP+0.1 cc. Auxilin	$\mathcal{Q}$	10	24.9 to 68.6	44.8±4.3
40 mg. AP (Subcutaneously)+0.1 cc. Auxilin (Intraperitoneally)	$\mathcal{R}$	11	17.2 to 60.4	36.1±4.9
	15-day series			
Control		23	17.6 to 50.5	27.0±1.5
40 mg. AP 5 to 14	$\mathcal{S}$	8	32.7 to 70.4	43.0±3.9
40 mg. AP+0.05 cc. Auxilin	$\mathcal{T}$	9	31.8 to 76.0	51.5±4.8
	72-hour series[1]			
Control	$\mathcal{U}$	48	3.1 to 8.5	5.4±0.17
3 R.U. Mare Serum	$\mathcal{V}$	10	5.2 to 10.8	7.1±0.60
10 mg. AP	$\mathcal{W}$	25	3.9 to 12.3	7.7±0.37
10 mg. AP+0.1 cc. Auxilin	$\mathcal{X}$	20	3.5 to 10.0	7.9±0.46
10 mg. AP+0.25 mg. Hemin+Pyridine	$\mathcal{Y}$	12	6.9 to 12.3	8.9±0.48
10 mg. AP+0.05 cc. H_2O Chlorophyll	$\mathcal{Z}$	14	5.8 to 12.5	9.3±0.48
10 mg. AP+0.05 cc. H_2O Chlorophyll+ Pyridine	2A	10	5.0 to 9.7	7.8±0.46
50 mg. AP	2B	12	6.2 to 12.4	9.9±0.56
50 mg. AP+0.1 cc. Auxilin	2C	9	7.1 to 15.8	11.6±0.75

[1] Single injection.

alone had an average comb factor ($\sqrt{H \times L}$ mm.) of 7.0, and the birds which were given auxilin with the pituitary extract had an average comb factor of 10.0. Since the comb factor is a linear representation (square root) this is a significant difference in size and indicates a definite physiological difference in the testes of the two series.

It has been pointed out that chicks kept without food and water for the 72-hour period just after hatching offer a more critical test for pituitary hormones. Unpublished data from our laboratory indicate the probable reason for this to be the lack of secretion of pituitary hormone from the chick's own pituitary gland under these conditions. The injection of 3 R.U. of pregnant mare serum, a preparation which is very potent in the male bird (series $\mathcal{V}$), produced testes which averaged 7.1 mg. in contrast to the 5.4 mg. control average, and this gives some measure of the degree of response

elicited. The data for the chicks in the 72-hour experiments demonstrated definitely that augmentation can also occur in the chick, and the results are essentially in accord with those observed for the rat. The water-soluble chlorophyll produced the most marked augmentation of any of the substances tested on the chick. When pyridine was added to the chlorophyll-pituitary mixture, the augmentation effect was not present, whereas the hemin-pituitary solution with pyridine increased the effect of the hormone. There is, therefore, a fundamental difference between hemin and chlorophyll with respect to their efficiency after the addition of pyridine.

A conception of the effectiveness of chlorophyll in increasing the action of the pituitary extract can be gained by observing that testes averaged 7.7 mg. after the injection of 10 mg. of hormone (series *W*), but when this same amount of hormone was combined with the chlorophyll, the mean weight increased to 9.3 mg. (series *Z*). The response elicited by 50 mg. of pituitary extract, on the other hand, was 9.9 mg. (series *2B*) and may be used as a measure of the augmentation. Auxilin, the only one of the plant hormones tested thus far on the chick, when combined with 10 mg. of pituitary extract (series *X*), did not increase the effectiveness of the hormone, but when added to 50 mg. the gonadotropic action of the preparation was augmented, and the testes averaged 11.6 mg. (series *2C*).

These data indicate that the action of pituitary gonadotropic extract can be augmented in both the chick and rat. Several problems are suggested, primarily with respect to the nature of the solution which results from the admixture of the various substances in vitro. The addition of the indole-butyric acid or the *l*-napthalene acetic acid changes the pituitary solution from a PH 7.9 to PH 5.3. Bischoff (5) demonstrated that addition of copper and zinc salts and modification of the PH resulted in augmentation and that change in the PH seemed to be the important factor in the reaction. He reported that maximum augmentation occurred at PH 8.5, near the precipitation point, and the least at PH 5.0. His results, therefore, are limited in their application to the experiments reported here for the plant growth hormone because augmentation occurred in the more acid solution, namely, at PH 5.3, and there was furthermore no optical indication of change in the nature of the solution nor any tendency to form a precipitate after the addition of plant hormones.

Dr. O. L. Inman of the Kettering Foundation was kind enough to analyze the chlorophyll samples used in this work. His analysis showed that in the alcoholic chlorophyll solution Cu had replaced the Mg in the molecule, but that the water-soluble preparation contained a monovalent metal probably Na or K, and likely the former. The significance of the presence of Cu in the mixture suggests that the augmentation which occurred with chlorophyll is similar to that described by Fevold et al. (6) and by Bischoff. The absence, however, of the Cu in the molecule of the water-soluble chlorophyll and the interesting PH variation noted previously in the plant growth hormone combinations indicates that the explanation for the augmentation in these series is probably too complex to explain on the basis of change in PH.

There can be little doubt, on the other hand, that combination with chlorophyll alters the physical state of the hormone, especially when the alcoholic chlorophyll was added. Whether the hormone is precipitated, adsorbed by the chlorophyll, or both, must be studied further, especially the effect of the alcohol in the one chlorophyll solution. That the physical nature of the solutions or mixtures can be analyzed is indicated by three things: the increase in the quantity of water-soluble chlorophyll from 0.05 cc. to 0.08 cc. per injection increased the effectiveness of the hormone; the response of the rat ovaries to various amounts of pituitary extract injected with a constant amount of alcoholic chlorophyll (0.05 cc.) was proportional to the amount of hormone used; and finally, separate injection of the hormone and chlorophyll as the augmentor did not result in augmentation, but rather in an inhibition of the pituitary action.

SUMMARY

The effectiveness of pituitary gonadotropic extract was increased in the immature female rat and certain chick experiments after admixture in vitro with alcohol or water-soluble chlorophyll, two of the plant growth hormones—indolebutyric acid and *l*-napthalene acetic acid—and hemin with pyridine added.

Chlorophyll, when injected separately from the pituitary extract, did not produce augmentation. Hemin, when injected with the hormone but without the pyridine added, likewise did not increase the gonadotropic action of the solution. The action of plant growth hormone when given separately was questionable. A total of 277 chicks and 137 rats was used in this study.

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AGE AND SEX DIFFERENCES IN HORMONE CONTENT OF THE RABBIT HYPOPHYSIS

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CONSTITUTIONAL FACTORS of age and sex are known to influence the expression of many spontaneous and experimental diseases. In a study of these factors, attention has been directed to the endocrine system. The following experiments were performed to obtain an index of the normal age and sex differences in endocrine function in one of the commonly used laboratory animals, the rabbit. Since the anterior hypophysis occupies a dominant position in the endocrine system, studies were confined to this gland. Estimates were made of the relative content, according to age and sex, of certain anterior lobe hormones of the rabbit hypophysis, based upon changes found in the thyroids, ovaries and adrenals of immature guinea pigs following pituitary implantations.

MATERIALS AND METHODS

The procedure was essentially that employed by Loeb in an analysis of anterior pituitaries of different species (1), and in a determination of differences in hormone content of the human anterior hypophysis dependent upon age and pregnancy (2). This method makes possible the analysis of individual hypophyses from mature rabbits, and small groups of glands from young animals. The results of preliminary experiments, using various amounts of tissue from mature male rabbits, were in agreement with the findings of Loeb for this species. Changes in the immature guinea pig ovary produced by gonadotropic hormones have been described in detail by Loeb (3).

All of the rabbits used were bred in a large animal colony, and the exact age and breed were known. Most of the animals used were hybrids, chiefly Belgian-English crosses, and a few Dutch and Himalayan hybrids. Others were of pure English, Belgian, and Dutch breeds. Including several supplementary experiments which were not tabulated, the pituitaries of 96 rabbits aged 10 days to 45 months were assayed, and 52 test animals were used, 46 of which received implants.

Pituitaries were obtained from freshly killed rabbits, immediately weighed, and kept on ice until used. Immature female guinea pigs, weighing from 170 to 210 gm. received subcutaneous implants of hypophyseal tissue daily for 4 days, and were killed for examination on the 5th day. Pituitaries from adult rabbits were analyzed by implantation of one quarter gland daily, and in the case of young rabbits, from one-half to two glands were used daily,

depending upon their size. In this way the amounts of tissue implanted in each comparable series were kept fairly constant. The posterior lobe was not removed before implantation, and division of the whole gland always included a portion of it in each fraction. A preliminary experiment showed that the presence of the posterior lobe would not appreciably affect the responses of the test animals to anterior lobe hormones.

At autopsy both ovaries and the left adrenal of each guinea pig were weighed and the gross appearance of the thyroid, uterus and vagina was noted. Following fixation in Petrunkevitch's solution, sections 4.5 μ in thickness were cut through representative areas of both ovaries, the left adrenal, and one thyroid lobe, and stained with hematoxylin and eosin. Conclusions were largely based upon examination of these sections.

The presence of large and mature follicles, usually associated with increased weight of the ovaries, was taken as evidence of the follicle stimulating hormone. Enlargement of the uterus and proliferation of the vaginal mucosa offered further evidence of the presence of active mature follicles. Luteinization of the theca interna around growing or atretic follicles, formation of interstitital gland, and the development of pseudo-lutein bodies gave an index of the amount of luteinizing hormone in the implanted tissue. Since there is no standard measure for these effects, arbitrary values based upon microscopic comparisons are given in the results (tables 1 to 5).

Changes in the thyroid are similarly designated. The gross appearance of the gland was seldom altered and microscopic changes were usually slight.

TABLE 1. CONTROLS: GROSS AND MICROSCOPIC FINDINGS IN 6 UNTREATED GUINEA PIGS

Guinea pig No.	Weight	Uterus and vagina	Ovaries			Thyroid stimulation	Left adrenal	
			Average wt.	Maturation of follicles	Leutinization		Weight	Mitoses per section
	gm.		mg.				mg.	
6	180	Thin, vagina closed	7.5	0	0	0	35	2
9	204	Medium, vagina closed	21	0	0	0	50	—
72	185	Thin, vagina closed	19	0	0	0	47	19
73	190	Medium, vagina closed	17	0	0	0	34	12
122	175	Medium, vagina closed	11	0	0	0	36	6
138	200	Medium, vagina closed	22.5	0	0	0	42	12
Average		Medium to thin, vagina closed	16.3	0	0	0	40.7	10.2

A single plus indicated a trace of hypertrophy, consisting in enlargement of some of the cells lining peripheral acini, together with the appearance of small vacuoles in the adjacent colloid. Higher values indicated greater activity.

Stimulation of the adrenal cortex was indicated partly by weight increase,

but chiefly by increased numbers of mitoses. The mitotic figures in three representative sections in each case were counted. In glands showing stimulation, mitoses were most frequent in the outer portion of the zona fascicularis.

RESULTS

a), *Controls*. Six normal female guinea pigs of the same weight and stock used in the experiments were examined at different times, and the findings are recorded in table 1. The uterus and vagina were not enlarged in any of

TABLE 2. IMPLANTATION OF HYPOPHYSES FROM YOUNG ADULT MALE RABBITS

Exp. No.	Rabbits used (all ♂)		Effects on the Guinea Pigs							
			Initial and final wt. of test animals	Condition of uterus	Ovaries			Thyroid stimulation	Left adrenal	
	Breed and age	Wt. of pituitary			Average wt.	Follicle maturation	Luteinization		Wt.	Mitoses per section
		mg.	gm.		mg.				mg.	
131	Belg.-Eng. 4 mo.	44	190–170	Enlarged vag. open	57	+++	++++	+	47	50
132	Hyb.-Eng. 4 mo.	26	185–175	Enlarged vag. open	116	++++	++++	+	73	68
133	Eng.-Hyb. 4 mo.	29	175–170	Enlarged vag. open	78	++++	++++	o	54	69
136	Belg.-Eng. 4 mo.	30	190–205	Medium vag. closed	33	±	+++	++	72	56
137	Belg.-Eng. 4 mo.	29	180–180	Enlarged vag. closed	51	+++	++++	+	73	39
143	Dutch 4½ mo.	27	185–210	Enlarged vag. open	34	++	++++	+	74	85
189	Belg.-Eng. 5½ mo.	23	170–185	Enlarged vag. open	77	++++	++++	+	54	23
190	Belg.-Eng. 5½ mo.	25	165–180	Enlarged vag. open	37	++±	+++	++	51	29
161	English 5½ mo.	21	185–180	Enlarged vag. open	78	++++	+++±	+	60	86
159	English 6 mo.	21	180–185	Enlarged vag. open	66	++++	+++	+	52	41
Average		27.5			62.7	+++	+++±	+	61	55.6

the cases, and the vagina was invariably closed. There was no gross or microscopic evidence of sexual maturity. The ovaries weighed from 7 to 27 mg. Microscopic examination showed the presence of growing follicles and a few large follicles which did not reach maturity in any case, but appeared to undergo atresia after reaching large size. The thyroid was never enlarged, and there was no microscopic evidence of stimulation. Rather flat epithelium surrounded solid colloid in peripheral acini. The left adrenals weighed from

34 to 50 mg., and mitoses per section varied from 2 to 20. In view of the uniform condition of these animals, the average values for this series were considered as normal standards of comparison for the effects produced by the implantations.

b), Effects of hypophyses from young mature rabbits of both sexes. Hypophyses from 10 male and 10 female rabbits were analyzed separately and the values obtained in each experiment are shown in tables 2 and 3. The rabbits

TABLE 3. IMPLANTATION OF HYPOPHYSES FROM YOUNG ADULT FEMALE RABBITS

Exp. No.	Rabbits used (all ♀)		Effects on the Guinea Pigs							
			Initial and final wt. of test animals	Condition of uterus and vagina	Ovaries			Thyroid stimulation	Left adrenal	
	Breed and age	Wt. of pituitary			Average wt.	Follicle maturation	Luteinization		Wt.	Mitoses per section
		mg.	gm.		mg.				mg.	
105	Belg.-Eng. 5 mo.	42	175–165	Enlarged vag. open	22	+±	++±	++	53	34
106	Belg.-Eng. 5 mo.	32	175–155	Enlarged vag. open	37	++±	++	+	60	20
110	Belg.-Eng. 5 mo.	38	180–165	Mod. enlarged vag. open	33	+++	++++	o	45	17
111	Belg.-Eng. 5 mo.	40	185–190	Enlarged vag. open	25	++	++	++	50	16
114	Belg.-Eng. 5 mo.	—	185–185	Enlarged vag. open	19	++	+++	+	53	6
194	Belg.-Eng. 5 mo.	54	190–195	Mod. enlarged vag. closed	27	+±	++±	o	50	29
192	Belg.-Eng. 5 mo.	40	170–195	Enlarged vag. closed	37	++	++++	++	66	13
119	Belg.-Eng. 5½ mo.	30	190–200	Enlarged vag. open	32	++	+++	+	59	20
162	English 8 mo.	33	185–205	Enlarged vag. open	18	+±	+++±	+	50	134
158	English 8 mo.	35	180–190	Enlarged vag. open	43	++±	++±	o	59	37
Average		38			29.3	++	+++	+	54.5	31.6

for the most part were English and Belgian-English hybrids aged from 4 to 8 months. On the average the males were slightly younger than the females, but in each instance sexual maturity was confirmed at autopsy. Virgin females were used, and none of the series showed evidence of pseudo-pregnancy when killed. The ovaries of these rabbits contained large clear follicles but no corpora lutea, and the uteri were usually in estrus condition. The males had not been used for breeding.

On the average, the pituitaries from female rabbits produced moderate

gonadotropic effects. The ovaries of the test animals were enlarged, and usually contained two or three large follicles, together with a few smaller growing follicles. The interstitital gland was moderately well developed and a corresponding degree of luteinization of the theca interna about growing and atretic follicles was observed. One or two pseudolutein bodies were found in 5 of the 10 cases. Follicular atresia was not more prominent than in the normal immature guinea pig ovary.

Implants of male pituitaries, despite smaller size, produced more intense gonadotropic effects. The ovaries of the test animals were greatly enlarged, and usually contained several mature follicles as well as growing follicles of all sizes. Luteinization of the theca interna of growing and atretic follicles was quite advanced, and the interstitital gland was well developed. Several pseudolutein bodies were present in 7 out of 10 cases.

Slight thyrotropic effects were noted in the majority of each group, and on the average there appeared to be no differences according to sex. From the tables it is apparent that a wide variation was obtained in the values for adrenal cortex stimulation. Male pituitaries, however, tended to produce a greater increase in adrenal weight and number of mitoses per section.

c), *Effects of hypophyses from immature rabbits of both sexes.* In this series 12 experiments were carried out. The hypophyses from small groups of rabbits of the same age, usually of the same sex, were analyzed together. Ages of the groups ranged from 10 to 73 days, and daily implants varied from 2 to ½ glands, depending upon size. The majority of the rabbits were Belgian-English hybrids, and litter mates were used whenever possible. A few were Dutch and Himalayan hybrids. Table 4 gives the values obtained in each experiment, and the sex of the rabbits used.

It will be noted that at 10 days of age the rabbit pituitaries exerted no gonadotropic effects, although some thyroid and adrenal cortex stimulation were produced. In addition to thyroid and adrenal stimulation, glands from 15 and 21-day-old rabbits produced some growth and maturation of follicles, although not in so great a degree as when glands from older immature rabbits were implanted. Pituitaries from 28-day-old rabbits exerted in addition a weak luteinizing effect, and glands from older animals up to 10 weeks of age produced both follicle stimulation and luteinization, more or less in proportion to increasing age. Luteinization processes, even following implants of 10-week-old glands, were not so intense as when glands from mature animals of either sex were implanted, and pseudolutein bodies were not found in any of the test animals of this group. When pituitaries from 4- to 10-week-old animals were implanted, growth and maturation of follicles were more marked than following glands from mature females, although in the absence of a comparable degree of luteinization, this apparent difference may not be significant. Where sexes can be compared, the male pituitaries were slightly smaller, and tended to produce somewhat greater gonadotropic effects. In all but two experiments the thyroid was stimulated, and on the average a greater thyrotropic effect was found than following implants of adult glands. Some adrenal stimulation was produced in each case, but was not correlated with age or sex.

d), Effects of hypophyses from old female rabbits. The hypophyses from 10 old female rabbits were analyzed in this group. The rabbits belonged to the same strains as the younger mature females and were aged from 23 to 45 months. In all instances the animals had been intensively bred and had sub-

TABLE 4. IMPLANTATION OF HYPOPHYSES FROM IMMATURE RABBITS

Exp. No.	Rabbits used			No. of pituitaries implanted daily	Effects on the Guinea Pigs							
	Age	Sex	Average wt. of pituitary		Initial and final wt. of test animals	Condition of uterus and vagina	Ovaries			Thyroid stimulation	Left adrenal	
							Average wt.	Follicle maturation	Luteinization		Wt.	Mitoses per section
175	days 10	♂ & ♀	mg. 4	2	gm. 170–190	Medium vag. closed	mg. 16	o	o	+	mg. 60	27
129	15	♂ & ♀	6	1	190–195	Enlarged vag. closed	23	+±	o	+	48	12
128	15	♂ & ♀	6	1	195–200	Enlarged vag. open	30	++±	o	++	65	20
178	21	♂	7	1½	180–195	Enlarged vag. open	22	+	o	+	78	52
179	21	♀	7	1½	185–205	Moderately enlarged vag. closed	14	±	o	++	62	32
78	28	♂	10	½–1	185–165	Enlarged vag. open	37	++±	+	+	70	31
23	35	♂ & ♀	12	1	190–190	Enlarged vag. open	75	++++	+±	o	58	27
99	41	♂ & ♀	14	½–1	188–170	Enlarged vag. open	53	+++	++	o	42	21
169	52	♂	15	½	175–175	Enlarged vag. open	56	+++±	++±	++	60	67
168	52	♀	18	½	170–160	Enlarged vag. open	52	+++	++	++	51	16
167	73	♂	19	½	175–160	Enlarged vag. open	62	++++	++	++	80	16
166	73	♀	23	½	182–170	Enlarged vag. open	44	+++	++±	++	45	18

sequently been infertile for periods of about 6 months prior to study. They were neither pregnant nor pseudopregnant when killed. The ovaries usually contained a few large follicles and the uteri in most cases were grossly similar to those of the younger females. On the basis of breeding history, the animals were considered to be reaching the end of the period of reproductive activity. In 2 cases where the hypophyses were weighed, the glands were heavier than those of younger mature females. The others were similar in size or occasionally somewhat larger.

The results of implantations are shown in table 5. It will be seen that the gonadotropic potency was approximately equal to that of the younger mature females. Growth and maturation of follicles, changes in the theca interna of growing and atretic follicles, and the development of interstitital gland were quite comparable, but in only 3 cases were any pseudolutein bodies produced.

TABLE 5. IMPLANTATION OF HYPOPHYSES FROM OLD FEMALE RABBITS

Exp. No.	Rabbits used (all ♀)		Effects on the Guinea Pigs							
					Ovaries			Thyroid stimulation	Left adrenal	
	Breed and age	Wt. of pituitary	Initial and final wt. of test animals	Condition of uterus and vagina	Average wt.	Follicle maturation	Luteinization		Wt.	Mitoses per section
		mg.	gm.		mg.				mg.	
97	Belg.-Eng. 23 mo.	—	160–180	Enlarged vag. open	31	++	++	+	44	23
82	Belg.-Eng. 23 mo.	—	180–175	Enlarged vag. open	21	++	++±	+	58	29
123	Belg.-Eng. 23 mo.	—	200–200	Enlarged vag. open	26	+±	++	0	65	157
124	Belg.-Eng. 25 mo.	—	185–195	Enlarged vag. open	37	+±	++±	0	55	58
121	Belg.-Eng. 25 mo.	—	175–180	Enlarged vag. open	21	++	+++	0	49	16
60	Belg.-Eng. 26 mo.	—	175–165	Enlarged vag. closed	50	+++	++++	+	57	131
181	English 35 mo.	48	190–205	Enlarged vag. open	54	+++	+++	±	78	24
44	English 35 mo.	—	175–185	Enlarged vag. closed	26	+	++±	0	54	9
41	English 36 mo.	—	180–190	Enlarged vag. open	23	++	+±	0	60	108
35	Belg.-Rex. 45 mo.	45±	185–188	Enlarged vag. open	28	++±	++++	0	65	59
Average					31.7	++	+++	±	58.5	61.4

The thyroid was affected in only 4 of the test animals, and in these instances stimulation was relatively slight, Adrenal stimulation occurred in all but one case, and was usually of a higher order than found after implanting glands from younger mature rabbits, although individual variation was greater. A comparison with the effects of pituitaries from old male rabbits was not possible.

DISCUSSION

The rabbit hypophysis is well suited to studies of gonadotropic hormone content by this method. The proportions of gonadotropic hormones in

glands of this species are such that implantation of small amounts of tissue (one-quarter gland daily from mature animals) permits the expression of both follicle stimulation and luteinization. Larger implants produce a predominantly luteinizing effect, masking to some extent the effects of follicle stimulating hormones. By way of contrast, the proportions of gonadotropic hormones in the adult human and bovine hypophysis are such that pure follicle maturation is seldom produced by implants of fresh tissue, regardless of size. Additional procedures, such as treatment with formalin or urea (4), or serial implantations (5), are necessary to bring out the complete follicle stimulating effects.

However, implants of the optimum size for gonadotropic effects produced a minimum of thyroid stimulation. This necessitated microscopical comparisons in all cases, and occasional supplementary experiments using larger implants were necessary to confirm questionable differences. In view of the sensitivity of the guinea pig adrenal to extraneous factors, all of the changes found in this gland can not with certainty be attributed to the action of a specific hormone. There is much evidence that the anterior pituitary has a stimulating effect on the adrenal cortex, independent of gonadotropic and thyrotropic effects (6), but the relationships are not at present sufficiently clear to warrant discussion.

These experiments present in a general way a composite picture of the content of certain hormones of the rabbit hypophysis, which differs both with respect to sex and age. Minor variations within the groups may in part be attributed to breed differences in the rabbits. While it was not possible to keep this factor constant in all cases, related breeds showing similar body characters were largely used. Preliminary studies have indicated that breed differences in pituitary hormone content may exist, but the age and sex differences described in this paper have been consistent in the breeds thus far studied.

The significance of the observed sex differences is obscure. In adults the proportion of follicle stimulating to luteinizing hormone seems to be approximately the same in the two sexes, the male pituitary having greater absolute quantities of each factor. It seems probable that we are dealing with differences in reproductive physiology, the nature of which is not suggested by this approach. These differences are manifest to a slight degree even in the immature rabbit, and it is of possible importance in this connection that the male rabbit has been found to reach maturity at a slightly earlier age than the female. Similar sex differences in gonadotropic hormone content have been noted in the rat by Evans and Simpson (7).

Differences between the mature and immature rabbit hypophysis are comparable to those observed in the case of the human hypophysis (2). In both species gonadotropic hormones are present in relatively small amounts before sexual maturity, whereas the thyrotropic hormone is present in equal or perhaps greater concentration at this time. This age difference in the rabbit is further accentuated by the use of larger implants. In 4 supplementary experiments one gland daily from 4- and 7-week-old rabbits was implanted.

A very marked thyroid hypertrophy resulted in each instance, but the ovaries of the test animals weighed only from 7 to 27 mg., compared with 37 to 75 mg. when half this amount of tissue was used. The degree of thyroid hypertrophy was even greater than found after implanting comparable amounts of adult tissue, which invariably produced greater follicle stimulation and luteinization. Large implants of young glands therefore seemed to inhibit growth and maturation of follicles.

To explain this inhibition, two possibilities are suggested. In the first place, an antagonism may exist between the thyroid and follicle stimulating hormones. However, it has been shown that implantation of pituitaries from children may bring about growth and maturation of follicles together with a marked thyroid hypertrophy, so that an antagonistic relation between these hormones does not necessarily exist. It is more probable, therefore, that another factor, such as 'atresin,' described by Loeb in the bovine hypophysis (8), is present in the rabbit hypophysis and that its action is manifest under these conditions. Atresia of follicles was not observed, but this factor may merely inhibit growth and development of follicles.

The results of implanting glands from older mature rabbits indicate that there is no appreciable change in the amounts of gonadotropic hormones with increasing age past sexual maturity. Similarly, human hypophyses showed no changes in this respect. There was, however, in the case of rabbits a tendency for the thyrotropic hormone content to diminish with age, and for the adrenal stimulating factor to increase.

SUMMARY

Age and sex differences in hormone content of the rabbit hypophysis have been studied by means of implantation into immature female guinea pigs.

The male pituitary produced greater follicle stimulation and luteinization than did the female, and also a greater stimulation of the adrenal cortex. Thyrotropic effects were approximately equal. Pituitaries from 10-day-old rabbits produced thyroid and adrenal stimulation but no gonadotropic effects, whereas at 15 and 21 days follicle stimulation was also produced. Glands from 28-day and older rabbits produced both follicle stimulation and luteinization, although of a moderate degree. The thyrotropic effects of comparable amounts of pituitary tissue from immature rabbits were greater than from mature rabbits. Glands from old female rabbits produced about the same degree of follicle stimulation and luteinization as young mature females. However, there was less thyroid stimulation and generally greater stimulation of the adrenal cortex.

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THE EFFECT OF CASTRATION IN ALBINO RATS ON NITROGEN, SULFUR, CHLORIDE, SODIUM AND POTASSIUM METABOLISM, AND ON CHANGES IN WEIGHT AND FOOD INTAKE

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THE STUDIES on the metabolism of nitrogen, sulfur, chloride, sodium and potassium presented in this communication form part of an extended investigation of the metabolic changes taking place in animals deprived of one or the other of the endocrine glands.

EXPERIMENTAL

Thirty-six male and female albino rats (Wistar strain) were used in the experiment. The rats were kept in groups of 3 in metabolism cages, as described in a previous publication (1). Eighteen rats were castrated before puberty at the age of 7 weeks. The gonads were removed from the remaining 18 animals after puberty, when they were 6 months old. These groups will be referred to hereafter as 'young' and 'adult' rats.

Urine and feces were collected twice a week. The urine was analyzed for total nitrogen (Kjeldahl), urea (2), ammonia (Folin) uric acid (3), creatine and creatinine (4), total sulfur (5), total and inorganic sulfates (Folin), chloride (modified Volhard-Harvey method), sodium and potassium (6). Feces were analyzed for total nitrogen, total sulfur (7) and chloride (open Carius method). Food was analyzed for total nitrogen, total sulfur, chloride, sodium and potassium.

The 'young' rats were studied for a control period of 4 weeks before castration and for 7 months afterwards. The 'adult' rats were observed for a control period of 4 weeks before removal of the gonads and for 4 months afterwards. The figures mentioned in the discussion are averages of the results obtained on all the animals. A typical protocol is presented in the tables, showing daily averages for one rat.

DISCUSSION

Nitrogen metabolism. Korenchevsky (8) found a decrease in nitrogen metabolism in castrated dogs, which is more pronounced in 'fat' than in 'thin' castrates. In our rats, which were castrated after puberty and which are comparable to his 'thin' dogs, since they do not gain weight abnormally, urinary nitrogen excretion also shows either a slight decrease or remains unchanged at about 30% of the intake. In animals which have been castrated before puberty, however, nitrogen metabolism follows a different pattern. Total urinary nitrogen excretion increases from 13% of the intake during the control period to a peak of 40%, 12 weeks after operation. After this high

point has been reached, excretion begins to decrease, and 7 months after castration it seems to be stabilized at 31% of the intake, an amount similar to that excreted by animals which have been castrated after puberty. The increase in urinary nitrogen excretion is accounted for by urea nitrogen. Fecal nitrogen excretion remains unchanged in both groups.

Opinion regarding the influence of castration on creatine metabolism is somewhat divided. Read (9) stated that the creatine metabolism of Chinese eunuchs is greatly deranged. If castration took place before puberty was reached, it induced a continuance into adult life of pre-pubertal creatinuria. Shen (10), on the other hand, found no creatine in the urine of eunuchs. Kochakian and Murlin (11) confirmed the results of Shen. They showed that the creatine which appears in the urine of castrated dogs is due to diet, and not to a metabolic disturbance caused by castration. When a creatine-free diet was fed, no creatine was present in the urine, nor was the creatinine output affected in any way. This finding is at variance with that of Schrire and Zwarenstein (12), who reported that castration of adult male rabbits caused a considerable increase in creatinine output. Our experiments on castrated rats agree with those of Shen, and Kochakian and Murlin. There is no change in either creatine or creatinine excretion.

TABLE 1. NITROGEN METABOLISM IN MALE RATS CASTRATED BEFORE PUBERTY (DAILY AVERAGE BY MONTHS)

Date	Total nitrogen							Urea N		NH_3 N mg.	Preformed creatinine mg.	Creatinine mg.
	Urine		Feces		Intake mg.	Retention						
	mg.	% of intake	mg.	% of intake		mg.	% of intake	mg.	% in take			
Control period 1937 Feb. 2–Mar. 1	36	13	52	20	271	183	67	24.1	67	1.0	1	1
[1]Mar. 3–29	124	36	70	20	343	149	44	93.0	75	1.6	2	1
Mar. 30–Apr. 26	112	34	66	20	330	152	46	96.9	86	2.4	4	2
Apr. 27–May 24	124	40	61	19	317	132	41	104.2	84	2.1	5	1
May 25–June 21	97	30	63	20	322	162	50	82.5	85	1.9	4	2
June 22–July 26	105	35	69	23	303	129	42	87.2	83	1.8	4	2
Aug. 24–Sept. 20	97	31	63	20	309	149	49	81.5	84	1.9	3	1

[1] Castrated March 2nd.

The effect of the removal of the gonads on nitrogen metabolism is more readily demonstrable during the period of most active growth. Later on adjustment takes place and at about 9 months of age approximately the same percentage of the nitrogen intake is excreted in the 'young' as in the 'adult' group. The influence of castration on nitrogen metabolism is apparent only

in the course of exogenous protein catabolism, since the increase in total urinary nitrogen excretion is completely accounted for by urea nitrogen. Ammonia, uric acid, creatine and creatinine show no change.

TABLE 2. SULFUR METABOLISM IN MALE RATS CASTRATED BEFORE PUBERTY (DAILY AVERAGE BY MONTHS)

Date	Total sulfur							Neutral S		Sulfates		
	Urine		Feces		Intake mg.	Retention				Inorganic		Ethereal mg.
	mg.	% of intake	mg.	% of intake		mg.	% of intake	mg.	% of urine S	mg.	% of urine S	
Control period 1937 Feb. 2–Mar. 1	1.8	9	5.1	26	19.7	12.8	65	0.4	22	1.2	67	0.2
[1]Mar. 3–29	3.9	16	7.5	31	24.0	12.6	53	1.0	26	2.4	62	0.4
Mar. 30–Apr. 26	6.4	27	7.8	32	24.0	9.8	41	2.4	38	3.6	56	0.4
Apr. 27–May 24	8.4	37	7.9	34	23.0	6.7	29	2.9	35	5.0	60	0.5
May 25–June 21	6.8	28	8.1	33	24.6	9.7	39	2.1	31	4.4	65	0.3
June 22–July 26	6.8	28	10.4	42	24.5	7.3	30	2.2	32	4.4	65	0.2
Aug. 24–Sept. 20	7.9	31	10.3	41	25.1	6.9	28	2.2	28	5.4	68	0.2

[1] Castrated March 2nd.

Sulfur metabolism. 'Adult' castrated rats do not show any disturbance in endogenous sulfur metabolism, such as we encountered in suprarenalectomized (13) and hypophysectomized (14) animals. The slight decrease in sulfur excretion, expressed in percentage of intake, parallels the excretion of nitrogen and occurs as a part of protein catabolism.

The removal of the gonads before puberty, however, produces a different course of sulfur metabolism. Total urinary sulfur excretion rises from 9% of the intake during the control period to a peak of 37%, 12 weeks after operation; and remains at 30%, 7 months after castration. This rise in the excretion of sulfur is not restricted to the kidneys as it is in rats which have been deprived of either suprarenals or hypophysis. The sulfur which is lost by way of the gut also rises steadily from 26% of the intake during the control period to 41%, 7 months after operation. The fact that the excretion of fecal sulfur, expressed in percentage of intake, rises while fecal nitrogen excretion remains unchanged indicates a definite disturbance in protein catabolism. The ratio of N:S retention changes from 14.3:1 during the control period—which is normal for muscle protein—to 22:1, 7 months after castration. According to Wilson (15) the nitrogen of some proteins can be stored independently of the sulfur, hence it follows that a sulfur-poor material may be retained in the tissues. That the disturbance in the ability to utilize sulfur

involves the endogenous sulfur metabolism as well as protein catabolism is shown by the increase in the excretion of neutral sulfur, which rises from 22% of total urinary sulfur excretion during the control period to a peak of 38%, 2 months after castration. Four months after operation it is still at a level of 31%, but after a period of 7 months adjustment begins to take place and the excretion of neutral sulfur returns to nearly normal proportions. The excretion of inorganic and ethereal sulfates, expressed in percentage of total urinary sulfur, remains unchanged.

TABLE 3. CHLORIDE METABOLISM IN MALE RATS CASTRATED BEFORE PUBERTY

Date	Urine Excret.		Fecal Excret.		Intake mg.	Retention	
	mg.	% of intake	mg.	% of intake		mg.	% of intake
Control period 1937 Feb. 2–Mar. 1	19.8	26	5.1	6	76.6	51.7	68
[1]Mar. 3–29	38.5	39	7.5	8	97.7	51.7	53
Mar. 30–Apr. 26	43.9	47	6.8	7	94.2	43.5	46
Apr. 27–May 24	48.0	53	7.3	8	90.2	34.9	39
May 25–June 21	41.1	45	8.2	9	92.2	42.9	46
June 22–July 26	41.9	48	14.5	16	87.8	31.4	36
Aug. 24–Sept. 20	36.3	40	10.4	11	90.1	43.4	49

[1] Castrated March 2nd.

Apparently the removal of the gonads before puberty disturbs the whole endocrine pattern of the organism, resulting in an entirely different type of sulfur metabolism. Once puberty has been reached, castration does not interfere with endogenous sulfur metabolism; the disturbance—if any—being restricted to protein catabolism.

Chloride, sodium and potassium metabolism. In 'adult' rats urinary chloride excretion drops slightly from 42% of the intake during the control period to 38% immediately after operation, and is still at a level of only 32%, 4 months after castration. Fecal chloride excretion expressed in percentage of intake remains practically unchanged.

Urinary sodium and potassium excretion also shows a slight drop after castration. Eight weeks after operation only 30% of the sodium intake and 32% of the potassium intake are excreted as compared to 38% of the intake of sodium and 40% of the intake of potassium during the control period. After this interval of 2 months normal levels are again maintained.

In the 'young' group urinary chloride excretion rises from 28% of the intake during the control period to 40% immediately after castration; and to 55%, 12 weeks after operation. Seven months afterwards it has returned to the initial value after castration; 41% of the intake.

Heller and Haddad (16) have shown that in normal rats the percentage of fecal excretion rises with the chloride intake. In our experiments on suprarenalectomized animals (17) this change in the path of excretion proceeded in the opposite direction, counteracting the increase in urinary excretion, so that the same percentage of the intake was retained as before suprarenalec-

tomy. In castration the excretion of chloride by way of the gut follows the same pattern as in suprarenalectomy, though to a lesser degree. Fecal excretion, expressed in percentage of total excretion, drops from 18% during the control period to 12%, 12 weeks after operation, then it rises again to normal proportions. After the peak of urinary excretion has been reached at 12 weeks after castration, fecal excretion, expressed in percentage of intake, rises somewhat, thus counteracting the decreased urinary excretion and keeping retention on a lower level for several months. The increase in chloride intake after castration, 21% over that during the control period, did not increase chloride storage, as found in normal animals by Heller and Haddad (16). In spite of the increased food intake chloride retention decreases from 66% of the intake during the control period to 37%, 12 weeks after operation. Then it begins to rise again till at 7 months after castration it has reached nearly the same value as that immediately following operation, about 50% of the intake. This decrease in chloride retention, though it develops much more slowly, is similar to that which we observed in hypophysectomized animals (14). There chloride retention drops abruptly after operation to one fifth of the amount which was stored during the control period—undoubtedly due to diuresis—and it is still only two thirds of the amount retained before hypophysectomy 11 weeks after operation. The decrease in chloride retention in castrated animals cannot be attributed to diuresis, such as occurs in hypophysectomy, since the urine output increases very little.

TABLE 4. SODIUM AND POTASSIUM METABOLISM IN MALE RATS CASTRATED BEFORE PUBERTY

Date	Sodium					Potassium				
	Urine Excret.		Intake mg.	Retention		Urine Excret.		Intake mg.	Retention	
	mg.	% of intake		mg.	% of intake	mg.	% of intake		mg.	% of intake
Control period 1937 Feb. 2–Mar. 1	12.3	25	48.4	36.1	75	8.7	20	42.8	34.1	80
[1]Mar. 3–29	22.1	36	61.4	39.3	64	18.0	33	54.2	36.2	67
Mar. 30–Apr. 26	26.1	44	59.1	33.0	56	21.9	42	52.2	30.3	58
Apr. 27–May 24	29.2	51	56.8	27.6	49	25.3	50	50.1	24.8	50
May 25–June 21	26.1	44	59.3	33.2	56	21.9	41	53.1	31.2	59
June 22–July 26	25.2	44	57.5	32.3	56	21.5	41	52.3	30.8	59
Aug. 24–Sept. 20	28.5	48	59.1	30.6	52	24.3	45	53.7	29.4	55

[1] Castrated March 2nd.

Urinary sodium and potassium excretion closely follows that of the Cl ion, sodium excretion increasing from 24% of the intake to 52% after 12 weeks, and being maintained at a level of 43%, 7 months after operation,

potassium excretion rising from 19% of the intake to 51%, and dropping to 44% during the same interval.

Influence on weight and food intake. The evidence for the influence of gonadectomy on the development of obesity is conflicting. Stotsenburg (18) stated that the growth curve for castrated male rats is similar to that of the normals. Slonaker (19) found that the castrates gained more weight than the controls. Evans and Simpson (20), on the other hand, noted weight losses or smaller gains in weight in the castrates. Van Wagenen (21) who extended the period of observation over that used by Stotsenburg found that in later life castrates do not show the same gain in or maintenance of weight as the controls. In our experiments the weight curves of normal and castrated male rats are similar up to the age of 30 weeks. Then the curve of the castrates flattens out, and at the age of 40 weeks the intact animals weigh 10% more. Food intake, expressed in gm. per 100 gm. body weight, is somewhat higher in the normal group up to a weight of 270 gm. After that both groups consume practically the same amount of food.

Regarding the female rat Stotsenburg (22), Slonaker (19), and Holt and co-workers (23) reported that oöphorectomized animals gain more weight than their controls. On the other hand, Schultze (24) found this increase in only 35% of his animals. Reed, Anderson and Mendel (25), and Cramer and Marshall (26) did not observe any marked tendency to deposition of fat in their castrated rats. Korenchevsky (8) in his experiments on dogs stresses the fact that gonadectomy produces two different types of castrates: 'fat' and 'thin' castrates. In those of our female rats which were castrated before puberty the weight curve is that of a 'thin' castrate and similar to that of an intact animal. These rats consume the same amount of food per 100 gm. of body weight as the controls. If castration is performed after puberty, however, the curve shows a definite gain in weight over that of the normal animals, which is maintained up to the age of 43 weeks, when the experiment was terminated. This gain in weight was achieved in spite of the fact that their food intake, expressed in gm. per 100 gm. of body weight was less than that of the normal group.

SUMMARY

The influence of castration on the metabolism of nitrogen, sulfur, chloride, sodium and potassium, and on weight and food intake of male and female albino rats was studied. If the gonads are removed after puberty nitrogen, sulfur, chloride, sodium and potassium metabolism either decreases slightly or remains unchanged. If castration occurs before puberty, however, urinary nitrogen excretion increases considerably. Urea nitrogen accounts for the increase. Ammonia nitrogen, uric acid, creatine, creatinine and fecal nitrogen excretion remain unchanged. Exogenous, as well as endogenous, sulfur metabolism is disturbed. Total sulfur excretion rises as well as that of neutral sulfur. After a period of 7 months endogenous sulfur metabolism begins to return to normal. Chloride, sodium and potassium retention is decreased. The weight curves and food intake of male and female rats which have been cas-

trated before puberty is similar to that of normal animals. The weight curves of male rats which have been castrated after puberty are similar to those of normal animals up to the age of 30 weeks. Then the curve of the castrates flattens out and at 40 weeks of age the controls weigh 10% more. Food intake, expressed in gm. per 100 gm. body weight, is somewhat higher in the normals up to a weight of 270 gm. Afterwards it is the same for both groups. The weight curves of female rats which have been castrated after puberty show a gain in weight over the controls, in spite of the fact that their food intake, expressed in gm. per 100 gm. body weight, is less. The significance of these changes has been discussed.

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PATENCY OF THE UTEROTUBAL JUNCTION OF THE RABBIT: EXPERIMENTAL OBSERVATIONS WITH THE AID OF CO_2 INSUFFLATION (RUBIN METHOD)[1]

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THE FOLLOWING experiments were undertaken to obtain a clearer understanding of the mechanical and physiological factors involved in the patency of the uterotubal junction of the rabbit, as determined by uterotubal insufflation. The special object of this study was to find an explanation of the non-inflammatory obstructions, so often encountered in clinical uterotubal insufflations. Though conditions in the rabbit are not entirely analogous to those in human beings, the degree of patency and the structure of the uterotubal junction are similar in many respects. The conflicting reports and theories so far presented warrant further study of this subject.

MATERIAL AND METHOD

The material for this study consisted of 48 mature rabbits, weighing from 2 to 4 kg. They were anesthetized by the intravenous injection of nembutal solution, 1 grain to 2.2 kg., and laparotomized. The genital tract was exposed, so that the exact time at which insufflated gas passed the uterotubal junction could be determined. A bulb-tipped medicine dropper or Keyes-Ultzman cannula was passed into the uterus through a small incision proximal to the uterotubal junction, and tied tightly. The pressures in mm. Hg at which CO_2 gas passed from the uterus into the tubes were recorded on the kymograph of the Rubin apparatus (1). The degree of patency of the uterotubal junction was also determined in retrograde fashion through a needle cannula tied in the fimbriated end of the tube. Carbon dioxide gas under 15 lb. of pressure was used at a constant rate of speed, 1 cc. per second.

Hormonic regulation of uterotubal patency. An attempt was made to determine whether the degree of patency of the uterotubal junction varied in typical fashion suggestive of a cyclical relationship to estrus, and to the cyclic changes which have been observed in the endometrium and myometrium of the uterus and tubes by Hammond and Marshall (2) and Moreaux (3).

In our experiments, the doe was considered to be in the anestrous stage if she did not accept the buck. As suggested by Friedman (4), estrous rabbits were obtained from healthy animals in the post-pseudopregnancy state. Pseudopregnancy was produced by the intravenous injection of urine from

[1] We are indebted to Dr. I. C. Rubin for the suggestion and supervision of these experiments.

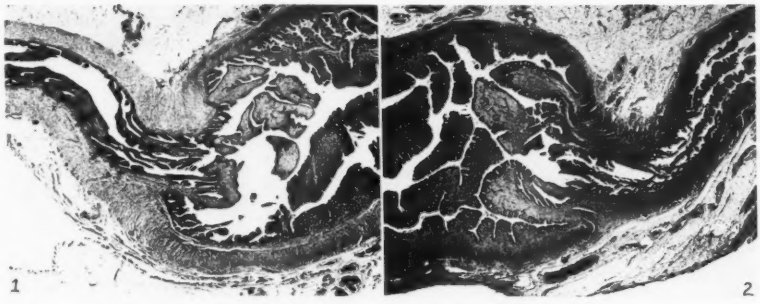

Fig. 1. Uterotubal junction of rabbit during pseudopregnancy.
Fig. 2. Uterotubal junction of rabbit in the estrous state. There are numerous polyp-like projections covered with tubal epithelium protruding into the uterine cavity. The musculature at the uterotubal junction is well developed.

gravid women. The presence of corpora lutea and corpora hemorrhagica was confirmed at laparotomy. Ovulation was observed at laparotomy, 10 hours after copulation.

At each stage of the estrous cycle we were able to corroborate the histological changes of the uterus and tubes, hitherto described. Throughout the estrous cycle we observed a marked thickening of the musculature and numerous polyp-like projections covered with tubal epithelium at the uterotubal junction (fig. 1, 2).

The pressures at which gas passed the uterotubal junction in 66 tests in living rabbits are recorded in table 1.

Three animals were insufflated immediately after they had died. In one of these, gas failed to pass either uterotubal junction at pressures of 220 mm. Hg. In one rabbit gas passed into the tubes at 60 mm. Hg. In the third rabbit, gas passed one uterotubal junction at 100 mm. Hg, but failed to pass the other at 168 mm. Hg. From these findings it is evident that there is a large variation in the degree of patency of the uterotubal junction at each stage.

In 22 of 66 tests in living rabbits pressures of 150 mm. Hg, or more were required to pass gas through the uterotubal junction. Maintaining a high pressure for a minute or two usually succeeded in overcoming the resistance. However, in two cases at anestrous, gas failed to pass into the tubes at pressures of 200 and 220 mm. Hg, despite maintenance of the gas pressure. In these two instances the site of obstruction was found to be located at the

Table 1. Uterotubal patency in relation to the estrous cycle

Pressures	Anestrus	Estrus	Ovulation	Pregnancy	Pseudo-pregnancy	Castration
mm. Hg	No.	No.	No.	No.	No.	No.
50 to 100	4	4	4	4		4
100 to 150	7	3	3	2	2	5
150 to 200	7	4	1	1	4	3
200 to 220	3				1	
Total	21	11	8	7	7	12

TABLE 2. REPEATED TESTS IN THE SAME RABBIT AT DIFFERENT STAGES

No.	Anestrus	Pseudo-pregnancy	Estrus	Castration	Ovulation	Pregnancy
	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg
1	198	185				
2	148	150	190			
3	90	132	130			
4		218	192			
5		165	195	120		
6			98	178		
7					74	78
8					78	168
9					100	103
10					125	88

uterotubal junction as excision of successive fragments of the tubes from the fimbria toward the uterus showed. Only after the junction was excised did the gas pass. No organic cause for the obstruction could be seen.

Repeated insufflations. Uterotubal gas insufflation was repeated in 4 rabbits during the anestrous stage. In one, the gas which had previously passed from the uterus into the tubes at a pressure of 100 mm. Hg, 3 weeks later went through only at a pressure of 170 mm. Hg. In another, gas passed the uterotubal junction at 98 mm. Hg, and 3 weeks later at 158 mm. Hg. In the 3rd rabbit, gas passed the uterotubal junction at 158 mm. Hg, and a week later at 150 mm. Hg. In the 4th rabbit gas passed into the tubes from the uterus at 200 mm. Hg, and 3 weeks later at 208 mm. Hg.

During pregnancy, gas passed the uterotubal junction of one rabbit at 70 mm. Hg. Five days after this animal aborted, gas passed from the uterus into the tubes at 105 mm. Hg.

Repeated insufflations at different stages of the estrual cycle have resulted in the findings shown in table 2.

From the above findings, we note that the degree of patency of the uterotubal junction bears no constant relationship to the estrous cycle.

Patency of the right and left uterotubal junctions. In 6 rabbits an opportunity was afforded to note the patency of each uterotubal junction in the same animal under identical conditions. Two cannulae were used, one introduced in each uterine horn and attached to a mercury manometer. The patency of each junction was noted at exactly the same time. The results are tabulated in table 3.

The patency of each uterotubal junction was also noted in 7 rabbits under

TABLE 3. PATENCY OF EACH UTEROTUBAL JUNCTION UNDER IDENTICAL CONDITIONS

Stage of cycle	Right junction	Left junction
	mm. Hg	mm. Hg
Estrus	175	173
Anestrus	90	90
Anestrus	153	118
Pregnancy	68	98
Pregnancy	121	148
Pseudopregnancy	250	165

TABLE 4. PATENCY OF EACH UTEROTUBAL JUNCTION WITH AN INTERVAL OF SEVERAL MINUTES ELAPSING BETWEEN EACH TEST

Stage of cycle	Right junction	Left junction
	mm. Hg	mm. Hg
Estrus	85	98
Estrus	92	78
Estrus	98	210
Pregnancy	78	55
Pregnancy	95	165
Pseudopregnancy	183	148
Death	98	168

the same conditions, except that several minutes elapsed between the insufflation of one side and the other. The results are tabulated in table 4.

Apparently, there is frequently a difference in patency of the two uterotubal junctions of the same rabbit. In no case was there any evidence of genital inflammation.

A similar variation in patency of each uterotubal junction has been noted by Dr. I. C. Rubin (5) in a woman with uterus didelphys.

Patency of uterotubal junction estimated from uterine end and from the fimbriated end. In 14 experiments, after the patency of the uterotubal junction was determined by insufflating gas through the uterus, it was also determined in the opposite direction by insufflating gas through the fimbriated end. For the latter purpose a fine cannula was used. The results are tabulated in table 5.

There is a striking difference in the degree of patency of the junction of uterus and tube depending upon the direction of the instreaming gas. The pressures are considerably less when insufflation is directed from the abdominal end of the tube downward toward the uterus. The polyp-like projections at the uterotubal junction offer a greater resistance to gas passing upward from the uterus.

Once the insufflated gas passed the uterotubal junction from the uterus into the tubes, there was a drop of pressure varying between 30 and 130 mm. Hg. This lower pressure level, always under 100 mm. Hg, then maintained itself as long as the gas was allowed to flow. Slightly higher pressures were maintained when high pressures were required to force gas through the uterotubal junction into the tube.

TABLE 5. COMPARISON OF PATENCY OF UTEROTUBAL JUNCTION AS DETERMINED BY UTEROTUBAL AND TUBOUTERINE INSUFFLATION

Stage of cycle	Uterotubal	Tubouterine	Uterotubal	Tubouterine
	mm. Hg	mm. Hg	mm. Hg	mm. Hg
Anestrus	118	23		
Estrus	175	37		
Estrus	78	55	92	88
Estrus	117	77	173	70
Ovulation	125	58	73	43
Pregnancy	148	77		
Pregnancy	98	54	68	65
Pseudopregnancy	200	78		
Pseudopregnancy	165	35	250	40

When CO_2 gas was insufflated into the uterus from the tubal side, the initial pressure required for it to pass the uterobutal junction was maintained with little change as long as the gas was allowed to flow.

DISCUSSION

The point of highest resistance to the flow of CO_2 gas through the uterus and tubes of the rabbit was always found to be at the uterotubal junction. Apparently, it is the latter which acts as a barrier to the abdominal cavity rather than the peristaltic force of the tubes in a direction toward the uterus, as claimed by Mikulicz-Radecki, Lueg and Nahmmacher (6).

We have noted as a constant finding the presence of well developed circular muscle and polyp-like projections at the uterotubal junction. This is in agreement with Lee (7) and Andersen (8) who found the uterotubal junction to be guarded by a sphincter-like apparatus analogous to the pyloric sphincter.

The resistance of the uterotubal junction was overcome in 22 instances by maintaining pressures of 150 to 220 mm. Hg, for one or two minutes. This resistance was undoubtedly due to muscular tone and not to the oblique entry of the tube into the uterus as suggested by Bondi (9). This action is explained by Kok (10) who states that the sacral autonomic system sends stimulating tonus impulses to the musculature which may be so strong that temporary spastic contraction of the ring muscle occurs.

SUMMARY

The uterus and tubes of 48 living rabbits were insufflated 66 times, employing CO_2 gas and the kymograph as in the Rubin test, in order to observe the tonicity of the uterotubal junction.

There was a large variation in the degree of patency of the uterotubal junction at each stage of the estrous cycle. Repeated insufflations during the same and different stages of the estrous cycle showed no correlated typical pressure patterns.

The degree of patency of the right and left uterotubal junction was frequently different when insufflated under identical conditions, or after several minutes had elapsed between the insufflation of either horn. Whether the pressures required to force gas through the uterotubal junction were low or high, once this ostium was opened by the force of the gas a more or less constant fluctuation level was maintained, always under 100 mm. Hg.

The uterotubal junction of the rabbit appears to be guarded by a sphincter-like apparatus and polypoid projections, which offer a greater resistance to gas passing upward from the uterus than downward toward the uterus.

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THE LOCAL EFFECT OF LUTEOHORMONE ON UTERINE MUCOSA OF THE INFANTILE RABBIT

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IT IS THE PURPOSE of this paper to demonstrate that the hormone from the corpus luteum, when administered locally and into the lumen of the uterine horn of an infantile rabbit, has a far more decided effect than when it is injected subcutaneously.

In order to convey directly the corpus luteum hormone into the infantile rabbit's uterus, previously prepared with follicular hormone, different methods may be followed, each giving different results: *a*), the hormone may be injected into the horn in the form of a solution in sesame oil, thus provoking an inevitable distention of the organ which will increase as the experiment progresses through succeeding days; *b*), the preparation may be injected under the peritoneum around the uterine horn. The action proved to be far more effective with the former technic than with the latter, as is shown by the findings in the different groups of animals.

The unavoidable distention produced when injecting hormone into the uterine horn seems to be a negative factor, inasmuch as the changes of the uterine mucosa are concerned, making it difficult to accurately evaluate the results, as such distention tends to bring about a disappearance of the mucous infolding. We have attempted to obviate such difficulties partly by employing concentrated solutions of hormone, and partly by fixing the material after voiding the horn.

Our experiments were carried out on infantile rabbits, whose weight ranged from 600 to 800 gm. and which were prepared for 8 days with follicular hormone (50 I.U.), the corpus luteum hormone being injected into the uterus on the 9th day, subserously or subcutaneously according to the group to which the animal belonged. The injection into the lumen of the horn was made following a ligature at the base of the horn.

METHOD

Sixty rabbits were divided into groups according to, *a*), the channel of injection; *b*), the dosage of the hormone; *c*), the distention of the uterine horn; and *d*), the time elapsing between the injection and the autopsy.

The mucous changes have proved to be more uniform, and therefore easier to appreciate when luteohormone was given,[1] the distention then

[1] We are very much indebted to the Schering Kahlbaum A. G. for providing us with the luteohormone used in our series.

being 0.1 cc. and the time of reaction 72 hours. Results are reported for the following groups.

a), One-half mg. of luteohormone, in a solution of 0.1 cc. of sesame oil, was administered into the uterine horn; the other horn received 0.1 cc. of sesame oil. The horn receiving the hormone showed a typical effect, as indicated both by the location of the changes and the characteristic cytologic picture of chorion and epithelium (fig. 1). Such an effect corresponded to a

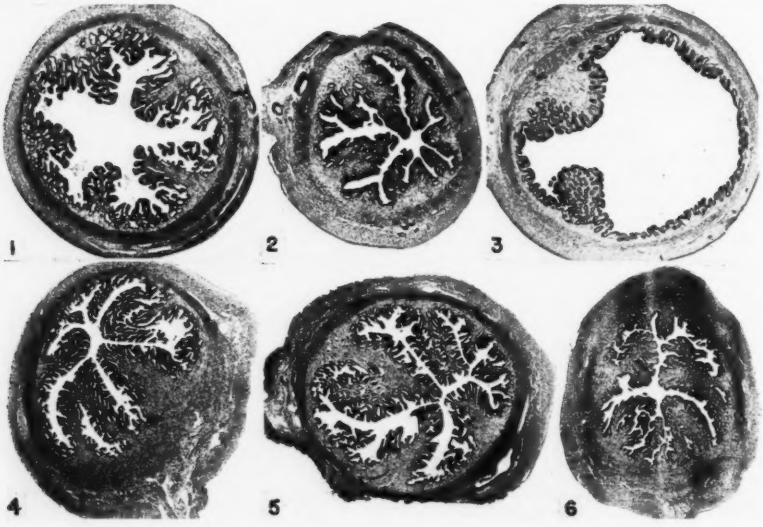

Fig. 1. 0.5 MG. LUTEOHORMONE IN 1 CC. Clauberg 3+. Local application. Fig. 2. HORN OF SAME GROUP AS THAT OF FIG. 1, BUT RECEIVING ONLY 0.1 CC. SESAME OIL. Clauberg +. Fig. 3. 0.5 MG. LUTEOHORMONE, SUBCUTANEOUSLY. Horn distended with 0.1 cc. sesame oil. Clauberg +. Fig. 4. CONTROL HORN FROM AN ANIMAL OF THE GROUP TO WHICH THE HORMONE WAS INJECTED ONLY INTO ONE OF THE HORNS. The one shown here was not distended. Fig. 5. 0.5 MG. LUTEOHORMONE SUBCUTANEOUSLY. Undistended horn. Clauberg 2+ to 3+. Fig. 6. HORN OF SAME GROUP AS THAT IN FIG. 5. Distended only with 0.1 cc. of sesame oil. Clauberg + to 2+.

Clauberg 3 + reaction. The action was also apparent in the horn receiving only sesame oil, but here the picture (fig. 2) was of a Clauberg + or 2 +.

b), One-half mg. of luteohormone in sesame oil was injected subcutaneously. Both horns were distended with 0.1 cc. of sesame oil. In general the picture was that of a Clauberg + or 2 + reaction (fig. 3).

c), One-half mg. of luteohormone in 0.1 cc. sesame oil was injected into one horn; the other received no injection. The cytologic changes were fairly uniform throughout this group. The transformation stage was visible in both horns, but there was an impression of a more intense effect in the injected horn, where the picture was of a Clauberg 3 + reaction, whereas in the other one (fig. 4) it was 2 +.

d), One-half mg. of luteohormone was injected subcutaneously. One of the horns received 0.1 cc. of sesame oil; the other remained untreated. The

effect was not uniform in this group, but a comparison between both horns in each animal showed that the undistended one had reacted more than the other. It may be stated that roughly the cytologic picture (fig. 5) in the undistended horn was that of a Clauberg 2+ to 3+ reaction and in the distended horn (fig. 6) a Clauberg + to 2+.

e), One-half mg. of luteohormone was injected subcutaneously. Both horns remained undistended. The reaction was fairly uniform in this group, and the cytologic picture was approximately that of a Clauberg 2+ reaction.

f), One-half mg. of luteohormone was deposited under the serous membrane of one of the horns, the other one receiving sesame oil. The cytologic pictures were those of a Clauberg + reaction in both horns. The results obtained are summarized in table 1.

TABLE 1

Group	Horn	Luteohormone subcutaneously .5 mg.	Luteohormone locally .5 mg.-0.1 cc.	Sesame oil	Clauberg reaction
1	A	—	Injected	—	+++
	B		—	Injected	+, ++
2	A	Injected	—	Injected	+, ++
	B		—	Injected	
3	A	—	Injected	—	+++
	B		—	—	++
4	A	Injected	—	Injected	+, ++
	B		—	—	++, +++
5	A	Injected	—	—	++
	B		—	—	

SUMMARY

The uterine injection of progesterone brings about a typical change in the mucous membrane of the injected organ. Such a change is more decided than when the same dose of corpus luteum is given subcutaneously.[2] Distention of the uterine horn seems to restrain the effect of luteohormone. The mucous changes are not so marked if the hormone is deposited under the peritoneum around the horn, as when administered into the uterus or subcutaneously.

[2] This increased activity of luteohormone, when injected into the uterus, leads us to assume that such a method of administration could be used as the one for the test of the hormone.

THE EFFECT OF SEX HORMONES ON THE COMBS OF CASTRATED AND NORMAL COCKS

DIRECT EFFECT OF FOLLICULIN ON THE COCK'S COMB

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IT HAS BEEN shown in previous papers that the follicular hormone (progynon-B), if applied locally on the comb of an uncastrated cock, brings about macroscopic and cytologic changes in both the comb and testes. Gross examination shows a striking reduction in size of the comb associated with pallor and flaccidity. Histological examination shows a marked diminution of the muco-elastic tissue, especially so at the apical end where it is wholly absent and where a dense connective tissue and an increased keratinization of the Malpighian epithelium are found instead. The testes, which are soft and paler than normal, show atrophy of the seminiferous epithelium together with a marked reduction of spermatogenesis and a definite diminution of interstitial tissue.

We have also demonstrated that the direct application of progesterone on the comb of an uncastrated cock produces neither macroscopic nor microscopic lesions in the testes or comb, and the cytological picture of these is undistinguishable from the normal. This hormone is ineffective as well on the castrated cock's comb.

Androsterone, however, when applied on the comb of an uncastrated cock causes it to enlarge considerably, along with a normal cytologic picture, and brings about deep changes in the testes as well. Our interpretation of these testicular changes determined either by the folliculin or the male hormone, is that they are due to a primary inhibition that checks hypophyseal function and goes together with secondary changes about the gonads, as has been the case in the experiments carried out on male and female mammals. We are studying at present the cytology of these hypophyses.

The routine technic generally employed in our experiments on cocks to determine the variation in the size of the comb, is as follows: the silhouette or outline of the comb is impressed on photographic paper, every effort being made so that both extreme ends of implantation of the comb on the head are clearly visible. The lamp is held at 40 cm. from the comb. The photographic outline thus obtained is then carefully redrawn on a paper of uniform thickness, and through both extreme points of implantation a line is drawn. The silhouette is carefully cut out and weighed by means of torsion scales. By comparing the weight of the cuttings obtained with the same comb before and after the experiment, the percentage of increase or decrease is estimated.

The variations in the size of the comb (in its area) is expressed in weight, according to the percentage of increase or decrease. The changes in the areas need not be actually measured, as they are estimated as related to its weight.

We have found that no increase in size was obtained by combining in vitro and in certain amounts the male hormone and folliculin, and then apply-

TABLE 1

Male Hormone Erugon 1 cc.=2 B.U.		Estrin 1 cc.=25 I.U.	Increase per cent	See Fig. 1
cc.		cc.		
.3	plus	1.7	0	1, 1a.[1]
.3	plus	1.5	0	2, 2a.
.3	plus	1.4	9	3, 3a.
.3	plus	1.3	14	4, 4a.
.3	plus	1.2	19	5, 5a.
.3	plus	1	22	6, 6a.
.3	plus	Oil	54	7, 7a.
Oil	plus	1.7	0	

[1] Number of photograph.

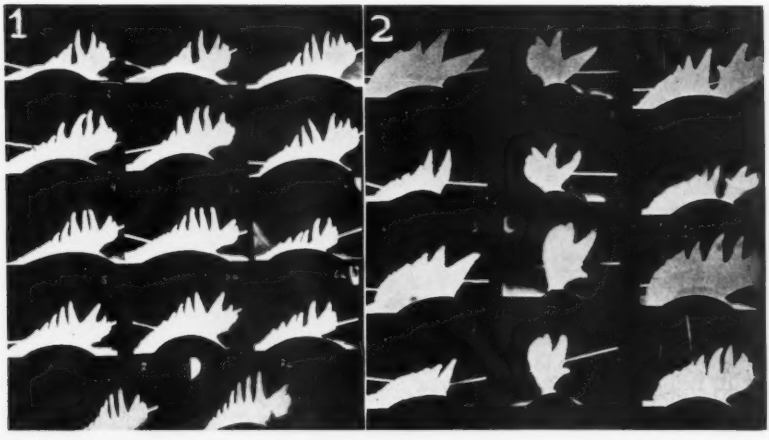

Fig. 1. ANTAGONISTIC EFFECT ON THE COMB, OF A COMBINATION IN VARIABLE AMOUNTS OF MALE AND FOLLICULAR HORMONES.

Fig. 2. DIRECT EFFECT ON THE DIVIDED COMB. Photos 1, 3, 5, and 7 were taken from left to right; photos 2, 4, 5 and 8 were taken in the reverse direction. 11–12, Effect of folliculin on the hen's comb.

ing such combination directly on the comb of a castrated cock. This negative result seemingly supported the evidence of a neutralization or antagonistic effect inasmuch as the cock's comb is concerned. This has been further corroborated in a larger series of animals.

The combination of 0.3 cc. of masculine hormone (erugon, 2 bird units per cc.) plus crystallized folliculin in oil solution (1 cc.–25,000 I.U.) absolutely failed to increase the size of the capon's comb, whereas erugon plus olive oil solution, brings about an average increase of 54 per cent. By reducing the

amounts of folliculin we have found that a moment comes when neutralization ceases to exist, although antagonistic effects still persist, as the masculine hormone alone being effective the increase is greater than in the presence of folliculin. (See table 1 and fig. 1.)

Our early estimations of the amounts of masculine hormone eliminated daily in the urine of normal human males and females, confronted us with a fact utterly adverse to Koch's results, as according to our findings the increase of the castrated cock's comb treated with urine of normal women was nearly one half less than the increase obtained with male urine.

This result was interpreted at the time as an inhibitory effect of folliculin toward the masculine hormone present in the urine, since our method of extraction yielded both hormones in the same preparation.

TABLE 2

Bird	Duration of exp.	Weight (mg.) of comb outline				Decrease %	
		Beginning		End			
254	10 days[1]	Ant. Portion folliculin 49.5 (1)	Post. Portion 30.5 (2)	Ant. Portion folliculin 26.8 (3)	Post. Portion 26.2 (4)	Ant. Portion folliculin 45.9	Post. Por. 14.1
273	10 days[1]	26.8 (9)	folliculin 24.2 (9)	24.0 (10)	folliculin 43.2 (10)	10.4	folliculin 45.5
273 Reversed experiment	11 days[1]	folliculin 48.0 (5)	37.5 (6)	folliculin 22.9 (7)	26.3 (8)	folliculin 52.3	29.9

[1] 18,750 I.U. of estrin were given daily in 2 doses.
Numerals in parentheses refer to the numbered photographs in fig. 2.

A series of experiments were carried out to make this clear, namely, the neutralization or antagonism in the combination made in vitro, and the reduced effect of the urine of normal women as compared with that of men.

The comb of an uncastrated cock was divided into two parts. The anterior one was given a local treatment with folliculin (progynon-B in oil), 25,000 I.U. twice a day for 15 days. A decrease was observed which was somewhat greater about the treated portion, anteriorly, than about the untreated one, posteriorly. The testes showed characteristic cytologic changes.

This result supports the view that folliculin has a direct effect, but in order to rule out completely any coincidence concerning the somewhat more marked decrease of the folliculin-treated portion, anteriorly, the same experiment was carried out on castrated cocks. With this in mind the combs of 2 castrated animals which had been employed for about 2 months for testing the masculine hormone, were divided. After the complete healing of the wound, both portions of each animal's divided comb were treated with androsterone and testosterone respectively, so as to obtain a total increase of over 150 per cent as compared to the initial size.

Folliculin was then applied anteriorly on the comb of one of the animals, and posteriorly on the other's comb. Notwithstanding the possible dissemination of the hormone throughout the body, and therefore the possible hormonic effect on the untreated portion of the comb, the results obtained were unquestionable. They are shown in table 2. The reduction of the comb was much more marked in the folliculin-treated portion than in the one decreasing as a consequence of the debilitating effect of the male hormone. Since the anterior portion increases and decreases in size more rapidly than does the posterior one, we reversed the experiment in one of the cocks (273) and found again that folliculin adds its effect to the spontaneous decreasing in size of the comb, thus a great difference appears in the experiments.

Decreases of 45.9, 45.5 and 52.3 per cent of the folliculin-treated portion of the comb, were obtained as against 14.1, 10.4 and 29.9 per cent of the non-treated portion spontaneously decreasing. We had at this point a reduced series of animals, but we think the results as shown in table 2 are so obvious as to rule out objections.

Special emphasis is laid upon this very important fact that goes to support the peripheral antagonistic effect of sex hormones without mediation of the hypophysis, which, as we know, is unquestionably involved in uncastrated animals.

This direct effect of folliculin on the cock's comb indicates the following conclusions. *a*), Extracts from organic fluids to be tested for male hormone (most frequently urine) must be completely freed from folliculin if accurate results are to be expected, for if such is not the case the estimation will only evidence the relative amounts of both hormones depending upon the antagonistic action. *b*), The estimations of these relative amounts might be perhaps of interest from the clinical standpoint, and should there be any deviation from the physiological normal standard either in men or in women, then the absolute estimation of each hormone separately could be performed.

These views considerably enlarge the field of investigation with reference to the clinical diagnosis of the disturbances of the genital system in males and females, as it is theoretically possible that an imbalance between the male and female hormones in the elimination stage (and perhaps in the production stage as well, though this is not so easily demonstrated) could be due to the increase or diminution of one of the hormones, while the other remains normal.

As early as 1935, during the course of our estimations of such relative hormonic amounts, we had succeeded in bringing to light the existence of a hormonic imbalance in the urine of women with universal hypertrichosis, evidencing itself in a percentage increase of the comb equal to that of the urine of men, and which sometimes was twice as marked as that of normal women. In such cases as these, several investigators (Koch among them) have shown in recent years that the absolute amount of male hormone was high as well.

It also seems possible that the absolute amount of the two hormones

could be either high or low without changing the normal physiologic balance between both of them, and that this could not be detected by the simple relative estimation with the capon's comb test.

The division of the comb into two portions made it possible for us to appreciate a fact already found by Fussgänger, namely, that the male hormone has a local and direct effect on the comb, which is greater than if the hormone enters the blood stream where it is either partially destroyed or goes through the elimination channels. Testosterone propionate was applied locally only on the anterior portion of the comb for a 15-day period (0.94 mg. daily in two doses and a total amount of 14 mg.); the increase thus obtained was 93.6 per cent in the anterior portion and only 52.1 per cent in the posterior one.

In spite of the deep testicular changes, the treated portion of the comb underwent a considerable enlargement (direct effect of the male hormone) while the posterior portion also increased although to a much lesser extent as a result of the hormone entering the circulation. Notwithstanding Tschopp's negative results with reference to the influence of folliculin on the comb of the hen, it would seem logical that also in this case the hormonic action might be similar to that obtained with the uncastrated cock.

We have just begun a series of experiments along this line, employing the White Leghorn hen to which folliculin is given for 15 days during the laying period (local application on the comb; 18, 750 I.U. daily in 2 doses). The size of the comb decreased 22.5 per cent and the organ became wrinkled and flabby (fig. 2, *11* and *12*). We are therefore in a position to support the claim of influence of folliculin on the hen's comb. Tschopp's negative results might possibly be accounted for as a consequence of insufficient doses.

SUMMARY

Previous experiments are confirmed concerning the neutral effects (inhibitory effects) of the combination of male hormone plus folliculin on the White Leghorn capon's comb. Folliculin has an influence on the uncastrated hen's comb that is similar, grossly, to the effects on the uncastrated cock's comb.

Folliculin has a direct effect on the cock's comb, just as previously reported experiments show. This opens a new field for experimental work in connection with the clinical use of the hormone.

Due emphasis should be laid upon our technic of dividing the comb, which is simple and easily performed.

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MINERAL SALTS AS TOXIC FACTORS IN URINARY PROLAN CONCENTRATES[1]

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DURING A recent investigation of the urinary prolan excretion of a non-pregnant human female (1), we experienced great difficulty in that when using the alcohol precipitation method and the original Aschheim-Zondek technic, the urinary concentrate proved toxic to many of the animals. Believing that the source of toxicity might be due to the mineral salts it contained, 3 specimens of the solutions of concentrates which had proven toxic were analyzed for various cations.[2]

The results were as follows; the metals being reported as the chlorides:

Soln. 1. ¼ of precipitate from 24-hr. specimen of urine, dissolved in 6 cc. H_2O.
KCl = 28.1 mg./cc.
Soln. 2. ¼ of 24-hr. specimen in 6 cc. H_2O.
$CaCl_2$ = 1.22 mg./cc.
Soln. 3. ¼ of 24-hr. specimen in 9 cc. H_2O.
NaCl = 35.10 mg./cc.
KCl = 15.83 mg./cc.
$CaCl_2$ = 0.20 mg./cc.
$MgCl_2$ = 0.07 mg./cc.

In order to determine the relation of these concentrations to the M.L.D., 21-day-old female rats were injected subcutaneously with solutions of pure salts. I believed it unwise to use the data in the literature on the toxicity of these salts for adult rats. Solutions were made up in the convenient strengths of 10% NaCl and 5% KCl, $CaCl_2$, and $MgCl_2$, and the dose was always diluted to 1 cc. for injection. The results and approximate time of death following the injections are as follows:

	M.S.D./kg.[1]	M.L.D./kg.[2]	M.L.D./30 gm.[3]	Time[4]
	gm.	*gm.*	*mg.*	*hr.*
NaCl	3.0	3.5	105	5
KCl	1.0	1.2	36	1
$CaCl_2$	0.7	1.0	30	15
$MgCl_2$	0.7	0.9	27	1

[1] Maximal safe dose per kg.: the largest dose given 5 rats where none died.
[2] Minimal lethal dose per kg.: the smallest dose given 5 rats where 3 or more died.
[3] Minimal lethal dose for a 30 gm. rat. The weight of 30 gm. was selected as the average weight of the 21-day rats we were using.
[4] Time: approximate time after injection of M.L.D. when animals died.

[1] Since completion of this paper, analysis of a composite of 5 urinary concentrates (first morning specimen in 9 cc. H_2O), which had proven toxic in doses of 1.0 cc. twice daily, gave the following results: Na (as NaCl) = 40.1 mg./cc.; K (as KCl) = 16.6 mg./cc. One specimen which was toxic in doses of 0.5 cc. twice daily, had the following surprising composition: Na (as NaCl) = 37.6 mg./cc.; K (as KCl) = 39.1 mg./cc.

[2] I am indebted to Dr. R. C. Neale for the analyses.

The amounts of Ca and Mg in solutions of the prolan precipitate are negligible so far as their M.L.D.'s are concerned. However, the amount of K per cc. in *soln.* 1 (28 mg.) is 28/36 of the M.L.D. for a 30-gm. rat, and the Na in *soln.* 3 (35 mg./cc.) is 35/105 of the M.L.D. Although both are below their M.L.D's, if given 3 times daily, as with the Aschheim-Zondek technic, it may well be understood why many animals die. Especially in the case of Na, where death occurs in about 5 hours, the effects can easily become cumulative. However, it seems most probable that it is the K which is responsible, since I have never noticed any hyperirritability typical of Na poisoning in rats dying during the injections of prolan concentrates. This agrees with the statement of Sollman (2) that 85% of the toxicity of the urine is due simply to its K salts. A point which may be of importance in explaining the death of the animals following repeated administration of sub-lethal doses is that they are often so depressed that they do not drink or eat.

Consequently, it appeared necessary to determine if the amount of K found in *soln.* 1 would prove toxic when administered 3 times daily for 2 days, and if a solution similar to *soln.* 3 would also prove toxic. Five female rats, 21 days old, weighing from 26 to 34 gm., were injected with synthetic *soln.* 1 (K) at 3-hour intervals, and 4 died, 3 of these after the third injection. Five more rats, weighing from 25 to 30 gm., were injected with synthetic *soln.* 3 (Na-K-Mg-Ca), at 3-hour intervals, 3 times daily for 2 days. Two of these rats died on the second day. In practically all cases, the smallest rats were the ones which died. This proves that the Na or K in the prolan concentrate is adequate to explain the toxicity, at least in part.

The possibility remained that in such a mixture of salts as in *soln.* 3, ion antagonism might occur, so that even if the M.L.D. of Na were administered, the Ca present might antagonize it. However, an attempt to demonstrate ion antagonism or synergism using adult mice, between certain ion pairs, as Na-K and Na-Ca, failed. The difficulty may lie in the fact that *a*), these salts may produce death by affecting different organs, *b*), their rates of absorption may differ, and *c*), that their time of death differs. For instance, administration of ½ the M.L.D. of Na occasionally prevents death by the simultaneous administration of an M.L.D. of K. This may be due merely to the fact that since both salts were administered in one solution, the hypertonic solution will so slow the absorption of the K that it can be excreted as rapidly as it is absorbed. Consequently, a fatal concentration will not accumulate. Because of these difficulties, the problem was abandoned.

The injection of NaCl alone, when determining the M.L.D., produced an amazing hyperirritability in the rats, probably due to what is mistermed Ca tetany. K, Mg, and Ca all produced depression, but rats dying of Ca showed strongly contracted hearts at autopsy.

Following a single injection of the approximate M.L.D. of 2 salts, it is usually easy to determine from which the animal dies, by the effects and the time of death. For instance, an animal injected with K and Ca—if it apparently dies of K poisoning—does so in an hour. If, not, it dies overnight with the strongly contracted heart typical of Ca poisoning.

The question as to how to diminish this toxicity of urinary concentrates may be solved in several ways, *a*), by using rats of a large strain weighing over 30 gm. at 21 days of age, *b*), by strictly spacing the injections at least 4 hours apart, to prevent cumulation, or better *c*), by taking up the precipitate in larger amounts of water in order to dilute the salts (as in 9 cc. instead of 6 cc.) and *d*), by giving injections twice daily instead of 3 times, or *e*), by previous removal of part of the salts, as by dialysis through Dupont cellophane tubing.[3] Application of principles *c*), and *d*) has proven very successful, in that many specimens of urinary concentrate solutions which were toxic with the Aschheim-Zondek technic, were injected without fatalities by using 9 cc. and 9 injections over 5 days, with but one exception out of 30 (1).

However, there are probably other toxic factors besides Na and K present in these urinary concentrates, such as the H^+ concentration.

SUMMARY

Urinary prolan concentrates made by the alcohol precipitation method contain sufficient amounts of Na and K to explain their toxicity. Suggestions for circumventing this toxicity are made. The M.L.D.'s of Na, K, Ca, and Mg chlorides for immature rats are given.

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FRACTIONATION STUDIES ON ADRENAL CORTEX EXTRACT WITH NOTES ON THE DISTRIBUTION OF BIOLOGICAL ACTIVITY AMONG THE CRYSTALLINE AND AMORPHOUS FRACTIONS

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IN A PREVIOUS publication from this laboratory (1) we described a practical method for fractionating adrenal glands to obtain an active cortical hormone extract suitable for parenteral injection in clinical cases of adrenal cortex deficiency. In the course of these studies we applied a rat method of assay which was studied in parallel with the dog method (2). The suitability of the adrenalectomized rat for evaluating the cortical hormone activity of adrenal extracts has received recent confirmation in the work of D'Amour and Funk (3).

The present report is concerned with the further chemical fractionation of our previously described extract in which a number of crystalline substances have been isolated analytically pure. Five of these appear to be identical with compounds previously described by Kendall et al., Wintersteiner and Pfiffner, and Reichstein et al. (4–11), and which have been identified as sterol derivatives (12–17).

The activity of these crystalline substances has been reported from several laboratories using widely different methods of assay (4, 10, 16), making comparison of relative activities difficult. Consequently, we have endeavored to evaluate the relative potencies of a number of these crystalline compounds using our standardized method of assay. The distribution of activity during the chemical fractionation has also been followed, permitting a quantitative estimate of the relative biological activities of the amorphous and crystalline fractions.

EXPERIMENTAL

Assay method. All fractions were assayed by the rat method previously described (2), the 'rat unit' having been defined as the minimum daily dose of hormone which, administered by single subcutaneous injection for 20 days to 4-week-old male rats weighing 50 to 60 gm., is sufficient to protect at least 80 per cent of the rats and produce an average growth of at least 20 gm. per rat for the 20-day period. The approximate value in 'dog units' can be obtained by multiplying the rat unit value by 22.

Starting material. The adrenal cortical extract obtained from 880 kg. of

beef adrenal glands by the previously published process was used in this investigation. The fraction weighed 20.96 gm. and assayed 4.5 R.U. per mg.

This material was practically free of water-soluble bases including salts of epinephrine. It contained no cholesterol or neutral fat. No solids could be extracted with petroleum ether from a 30 per cent methanol solution of this material.

Fractionation of the biologically active material. In preliminary distribution studies with small amounts of extract (10 kg. of gland), it was determined that the active fraction could easily be extracted from water with ethyl acetate, but that ether and benzene extract the hormone from aqueous phases with much more difficulty. Methods of purification based upon the distribution of the hormone between aqueous acid and ether, and aqueous bicarbonate and ether, have been used (18), and it was demonstrated that acidic and basic substances could be removed in this way with very little loss of activity.

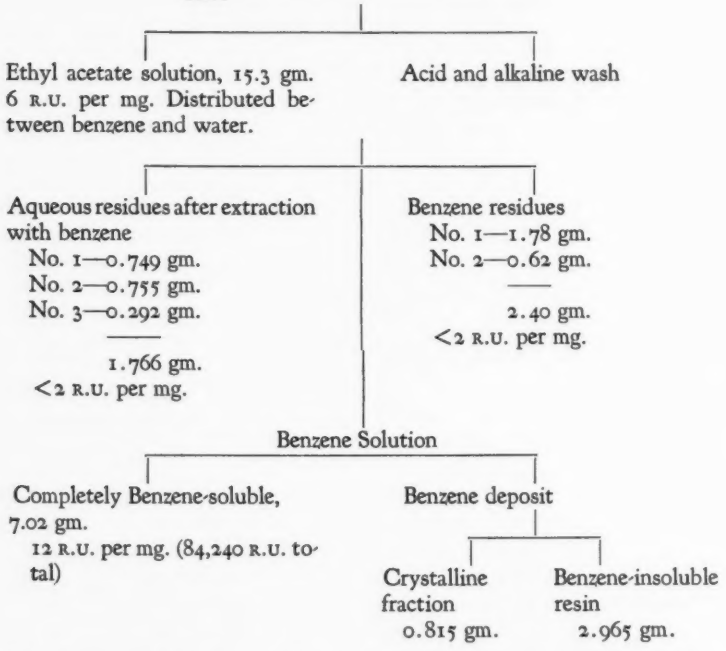
Because of the easy solubility of the active fraction in ethyl acetate, we have found that the acidic and basic substances can be removed by washing an ethyl acetate solution with an aqueous alkaline solution followed by an acid solution as has been mentioned by Reichstein (7). For an aqueous alkaline solution a sodium carbonate-sodium bicarbonate mixture of pH 8.5 was used, since washing with solutions of greater alkalinity resulted in loss of hormone. 0.5N HCl solution was used for an acid wash. In our hands this method has worked better than the longer procedure of Pfiffner et al. (18).

The active fraction obtained after separation of acidic and basic substances was distributed between benzene and water after the manner described by Mason, Myers and Kendall (6). The ethyl acetate solution was concentrated in vacuo at 45° and a gummy residue obtained which was only partially soluble in benzene. An aqueous suspension was obtained by dissolving the substance in 50 per cent ethanol and removing the ethanol in vacuo. This aqueous solution of 200 cc. was extracted with ten 200 cc. portions of pure benzene. A clear light yellow benzene solution, an aqueous residue, and a dark resinous substance insoluble in either benzene or water, were obtained. The benzene solution on standing at room temperature over night deposited a white semi-crystalline precipitate and more resinous material. The precipitated substance was removed by decantation and washed with two 200 cc. portions of benzene. The combined benzene solution was then concentrated to a volume of 200 cc. and exhaustively extracted with ten 200 cc. portions of water. This aqueous solution was now again concentrated in vacuo at 45° to 200 cc. and exhaustively extracted with benzene as above. Finally, this benzene solution was concentrated and extracted with water and the aqueous solution once more extracted with benzene.

There was thus obtained by this distribution: *a*), a water-soluble fraction, *b*), a benzene-soluble fraction that could not be extracted from benzene with water, *c*), a fraction extractable from benzene with water and from water with benzene, and *d*), a semicrystalline and resinous precipitate from the first benzene solution. The distribution of activity by this procedure is given in outline 1.

Outline 1

PRELIMINARY FRACTIONATION


20.96 gm. gland extractives 4.5 R.U. per mg. (94,320 R.U. total). Acidic and basic substances removed by washing with Na_2CO_3 and $NaHCO_3$ solution at pH 8.5, followed by 0.5N HCl
Ethyl acetate solution, 15.3 gm. 6 R.U. per mg. Distributed between benzene and water.
Acid and alkaline wash
Aqueous residues after extraction with benzene
No. 1—0.749 gm.
No. 2—0.755 gm.
No. 3—0.292 gm.
1.766 gm.
<2 R.U. per mg.
Benzene residues
No. 1—1.78 gm.
No. 2—0.62 gm.
2.40 gm.
<2 R.U. per mg.
Benzene Solution
Completely Benzene-soluble, 7.02 gm.
12 R.U. per mg. (84,240 R.U. total)
Benzene deposit
Crystalline fraction
0.815 gm.
Benzene-insoluble resin
2.965 gm.

The 815 mg. of crude crystallizate from the benzene solution (outline 1) was further fractionated and found to be a mixture of cube- and needle-shaped crystals. These crystalline fractions melted from 220° to 240°. By extensive fractional crystallization from dilute acetone, acetone, and alcohol, 3 fractions of constant melting point were finally obtained. The cube-shaped crystals were least soluble in acetone and could therefore be separated by repeatedly recrystallizing the first crop from acetone. There were fine needles that were very insoluble in 95 per cent ethyl alcohol and were therefore isolated by repeated recrystallization from this solvent. Finally, from the acetone mother liquors after separation of the above substances, small amounts of heavier needle crystals separated which were much more soluble both in acetone and alcohol than either of the other two.

The first of these substances (cube-shaped crystals from acetone) had a melting point of 241–245° (corrected) with decomposition, which could not be raised by further recrystallization from acetone. The substance was

Outline 2

FRACTIONATION OF COMPLETELY BENZENE-SOLUBLE SUBSTANCE WITH GIRARD'S REAGENT T.

7.02 gm. assaying 12 R.U. per mg. Reacted with Girard's reagent and extracted with ether at pH 7.5–8.0

Aqueous solution acidified to pH 6.0 with 0.5N HCl and extracted with six 500 cc. portions of ether.	Fraction No. I Non-ketonic portion 1.899 gm. 4 R.U. per mg.
Aqueous solution acidified to pH 4.0 with 20% tartaric acid and extracted with six 600 cc. portions of ether.	Fraction No. II 0.209 gm. 5 R.U. per mg.
Aqueous solution acidified to pH 2.0 with conc. HCl and extracted with six 700 cc. portions of ether.	Fraction No. III 1.337 gm. 15 R.U. per mg.
Aqueous solution further acidified with 1/10 volume of conc. HCl and extracted with six 700 cc. portions of ether.	Fraction No. IV 2.31 gm. >15 R.U. per mg. <20 R.U. per mg.
Aqueous acid residue discarded.	Fraction No. V 0.244 gm. 5 R.U. per mg.

optically active and the specific rotation was taken in absolute alcohol. There was obtained 53 mg. of the substance analytically pure.

Specific rotation: $[\alpha]_D^{25} = +70.5°$ (c = 0.674 in absolute EtOH).
Analysis by micro-combustion gave: C = 69.45, H = 8.41.
Calculated for $C_{21}H_{32}O_5$ C = 69.18, H = 8.86.

This substance is probably identical with compound *D* of Reichstein (5). It was assayed at a level of 2 R.U. per mg. (see substance V, Table 1) and found to be practically inactive at this dosage.

The needle crystals slightly soluble in alcohol had a melting point of 250–252° (corrected) with decomposition, which could not be raised by further recrystallization. Only 24 mg. was obtained analytically pure.

Specific rotation: $[\alpha]_D^{25} = +84.5°$ (c = 0.236 in absolute EtOH).
Analysis by micro-combustion gave: C = 68.79, H = 9.58.
Calculated for $C_{21}H_{34}O_5$: C = 68.80, H = 9.36.

These data agree with the data reported by Reichstein for his compound C

except that the rotation given by him for the *D* line in the same solvent is +69.8.° This substance was injected at levels of 2 and 8 R.U. per mg. (see substance IV, table 1) and found to assay approximately 3 to 4 R.U. per mg. This is interesting in view of the fact that Reichstein considered compound C to be inactive (19).

The heavier needle crystals more soluble in acetone and alcohol were recrystallized from acetone until a constant melting point of 231–234° (corrected) with decomposition was obtained. There was obtained 42 mg. analytically pure.

Specific rotation: $[\alpha]_D^{25} = +71.4°$ (c=0.28 in absolute EtOH).
Analysis by micro-combustion gave: C=68.38, H=9.15.

These analytical data agree quite well with the values for formula $C_{21}H_{34}O_5$. The melting point, specific rotation, solubility and crystalline form indicate that this compound is definitely different than either of the other two. Since the analytical data indicate the formula $C_{21}H_{34}O_5$, the possibility exists that we have here an optical isomer of compound C of Reichstein. This unidentified substance (see table 1) when assayed at a level of 2 R.U. per mg., was found to be inactive.

The benzene solution (outline 1), after separation of the crystalline and resinous precipitate, contained 7.02 gm. and this material passed well when assayed at a level of 12 R.U. per mg. and failed when assayed at 15 R.U. per mg. A threefold purification of the cortical hormone was therefore obtained at this stage, with an 89 per cent yield. This most active fraction was fractionated further with the use of Girard's reagent *T*, which was synthesized and used as described by Girard (20). Reichstein (7, 9) and Wintersteiner and Pfiffner (5) have made extensive use of this reagent in the isolation of crystalline substances and it was used here in a manner very similar to that described by them.

The benzene solution was concentrated in vacuo at 40° to remove all solvent, and the dried, straw-colored glassy residue was dissolved in 80 cc. of absolute methyl alcohol. The reaction with Girard's reagent was done in two equal portions on subsequent days. To each 40 cc. portion, 4 gm. of Girard's reagent and 2 cc. of glacial acetic acid were added and the mixture allowed to stand at room temperature for 2 hours. With the flask in an ice bath, 38 cc. of normal sodium carbonate solution was added and the methanol removed in vacuo at 0°. The pH of the resulting aqueous solution for the first portion was 8.0 and for the second portion 7.5. At this slightly alkaline pH the solution was thoroughly extracted with ether to remove the fraction that did not react with the reagent at room temperature. The ketones that reacted with the reagent were then fractionated by extracting with ether at pH 6.0, 4.0, 2.0 and finally at pH of 1N HCl. The ether extracts of the acidified solutions were washed free of acid by passing through a second separatory funnel containing 15 cc. of the sodium carbonate-sodium bicarbonate solution of pH 8.5. Each ether solution was concentrated and dried with anhydrous Na_2SO_4. The water separating on concentration was added to the aqueous solution before adjustment to the next lower pH. Water was recovered from the Na_2SO_4 by extracting with methanol and removing

methanol in vacuo. This aqueous portion was also added to the main aqueous solution before lowering pH for the next ether extraction. The summary of the distribution with yields of hormone obtained at each step is given in figure 2.

Fractions II to V (outline 2) were separately dissolved in pure chloroform and concentrated to syrupy residues in vacuo over calcium chloride and paraffin; the concentrates diluted with more chloroform and again concentrated as described by Wintersteiner and Pfiffner (5). Crystalline precipitates were obtained with fractions IV and V, but by far the greatest amount was obtained in fraction IV. The chloroform-insoluble substance was dissolved in 95 per cent ethyl alcohol for recrystallization; 364 mg. of crystals that melted at 201–210° (corrected) were obtained. Two more recrystallizations from 95 per cent ethyl alcohol yielded a top fraction of 170 mg. Beautiful rhombohedra were obtained from both alcohol and acetone and melted at 215–218° (corrected) with decomposition.

Specific rotation: $[\alpha]_D^{25} = +195°$ (c = 1.89 in 95% EtOH).
Analysis by micro-combustion gave: C = 70.05, H = 7.71.
Calculated for $C_{21}H_{28}O_5$: C = 69.96, H = 7.83.

This compound is undoubtedly identical with substance *E* of Kendall (8), *F* of Wintersteiner and Pfiffner (5) and *Fa* of Reichstein (9). In agreement with Kendall and Reichstein we found that the substance possesses biological activity. Our assays (see substance, I table 1) indicate approximately 1 R.U. per mg. This, however, is an activity only one-fifteenth as great as is contained in the amorphous fraction from which this substance was crystallized. By further distribution of fraction IV of outline 2 between benzene and water, the activity could only be increased to 20 R.U. per mg. Smaller fractions assaying 30 R.U. per mg. have been obtained by us.

The other fractions obtained, as represented in outline 1 were also further separated into ketonic and non-ketonic portions with Girard's reagent *T*. The 2.965 gm. of benzene-insoluble resin was reacted with the reagent and the non-ketonic portion extracted at an alkaline pH as above. The ketonic portion was hydrolyzed from the Girard's reagent at pH 2.0. The ketonic fraction weighed 1.212 gm. and the non-ketonic 1.33 gm.

The ketonic fraction was dissolved in pure acetone and the solution concentrated to 2.5 cc. After standing at −10° for two days a crystalline deposit was obtained. Further quantities of crystals were obtained by concentrating the mother liquor. The total first crude crystallizate from this fraction weighed 250 mg. and melted at 200–207°. It was recrystallized from acetone and finally from 95 per cent ethyl alcohol. A top fraction of 68 mg. was obtained analytically pure and melted at 216–218° (corrected), with decomposition.

Specific rotation: $[\alpha]_D^{28} = 164°$ (c = 0.68 in 95% EtOH).
Analysis by micro-combustion gave: C = 69.56, 69.47 H = 8.49, 8.38
Calculated for $C_{21}H_{30}O_5$: C = 69.57 H = 8.35.

This compound is undoubtedly identical with substance *M* of Reichstein (10).

This substance (II, table 1) was also found to be definitely biologically active, but contains slightly less than 2 R.U. per mg. The 3 rats used for assay of this substance were all living on the 20th day but had not gained an average of 20 gm. over the 20-day period. It possesses therefore the same order of cortical hormone activity as compound *E* of Kendall.

The 2.4 gm. of benzene most soluable fraction (the benzene residues of outline 1) were separated with Girard's reagent into ketonic and non-ketonic fractions in the same manner as the resin insoluble in benzene. The non-ketonic fraction weighed 0.523 gm. and the ketonic fraction 1.55 gm. Crystallization of this ketonic fraction from various solvents was unsuccessful. It was therefore fractionated with Girard's reagent into two fractions. The first was extracted with ether after acidification from pH 7.5 to 3.5 and the second fraction was extracted with ether after acidification to pH 1.5 in the first fraction 0.955 gm. was obtained and 0.567 gm. in the second. Although no crystallization occurred in the first with various solvents, the second fraction, after long standing (10 days) in isopropanol at $-10°$, yielded a crude crystallizate of 139 mg., which melted at 164–172° (corrected). Two recrystallizations from acetone yielded 46 mg. of heavy prisms that melted at 178–180° (corrected).

Specific rotation: (in 95% EtOH for both sodium and mercury lines)
$[\alpha]_D^{25} = +258.2$ (c=0.306 in 95% EtOH)
$[\alpha]_{5461}^{25} = +297.4$ (c=0.306 in 95% EtOH).
Analysis by micro-combustion gave: C=73.47, H=8.68.
Calculated for $C_{21}H_{28}O_4$: C=73.21, H=8.20.

There can be little doubt that this substance is identical with compound *A* of Kendall et al. (16), which Reichstein obtained by reduction of his corticosterone and termed "dehydro-cortico-sterone" (10). Steiger and Reichstein (21) have stated that corticosterone and dehydro-corticosterone have similar cortical hormone activity, which is greater than the hormonal activity of any of the other crystalline substances. Kendall et al., whose com-

TABLE 1. ASSAY OF CRYSTALLINE SUBSTANCES

Crystalline substances	Daily dose	No. of rats injected	No. of rats surviving on 20th day	Average growth in 20 days	Average survival[1]
	mg.			*gm.*	*days*
I. Compound *E* of Kendall	1.0	3	3	24	24
II. Substance *M* of Reichstein	0.5	3	3	16	29
III. Compound *A* of Kendall (Dehydro-corticosterone)	0.5	3	2	28	26
	0.125	3	0	—	13
IV. Substance *C* of Reichstein	0.5	3	3	27	31
	0.125	3	0	—	10
V. Substance *D* of Reichstein	0.5	3	0	—	10
Unidentified substance	0.5	3	0	—	9

[1] Injections were discontinued on 20th day.

pounds *A* and *B* are respectively identical with dehydro-corticosterone and corticosterone, have likewise stated that they are of similar activity (22). From the assay data available (see substance III, table 1) we estimate the

activity of dehydro-corticosterone, isolated as here described, to be approximately 3 to 4 R.U. per mg.

The summary of the assay results on the crystalline substances is given in table 1. Crystals were dissolved in 95 per cent ethyl alcohol and diluted with water to make a 10 per cent alcohol solution. The solution was administered in single daily doses of 0.5 cc., the concentration having been adjusted to give the required amount of substance in the 0.5 cc. dose. The average survival of uninjected controls is 7 days (2). Although limited amounts of the crystalline fractions made it impossible to run as many rats as would be desired, nevertheless, the results obtained make possible a fairly accurate estimate of the order of activities of these substances.

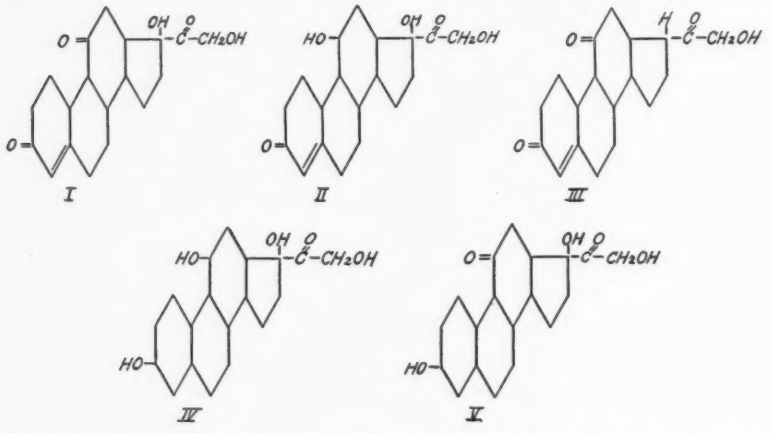
DISCUSSION OF RESULTS

Due to the work of Reichstein and of Kendall et al., to which we have referred above, the crystalline compounds isolated by us from the ketonic fractions and found to be identical with compounds isolated by them, can be represented by formulas I to III.

By our method, I and II were found to contain 1 to 2 R.U. per mg., whereas substance III assays approximately 3 to 4 R.U. per mg. The compounds isolated by the Kendall group were assayed in adrenalectomized rats by the muscle contraction test of Ingle (23). Reichstein also evaluated 'cortin' activity by a similar muscle contraction test (24). Both investigators reported that dehydro-corticosterone and corticosterone were practically equally biologically active, and that the compounds represented by formulas I and II were only half as active. Since they report that from 0.8 to 1.0 mg. of corticosterone or dehydro-corticosterone is necessary per rat per day in the muscle contraction test, it is apparent that larger doses are necessary to maintain a rat with a continuously contracting muscle than are necessary for the maintenance and growth required by our test. Grollman (25) has recently questioned whether these compounds actually contained any cortical hormone activity, since the muscle contraction test was not considered adequate by him. By the use of our prolonged 20-day rat test we have shown that, when injected in sufficient quantities, these compounds can substitute for adrenal cortical hormone solutions. Our results therefore confirm and extend the observations of both Kendall and Reichstein regarding the 'cortin' activity of these α, β-unsaturated sterol ketones.

Substances *C* and *D* of Reichstein have been shown by him to be closely related sterols represented by formulas IV and V. By chemical degradation he has thus far not been able to demonstrate which substance is represented by IV and which by V. Our analytical data for the substance, which we believe to be identical with substance *C* of Reichstein, agree very well with the values for formula IV, $C_{21}H_{34}O_5$. Reichstein's analytical data, as well as our own, also indicate formula V, $C_{21}H_{32}O_6$, for substance *D;* IV and V differ therefore from II and I respectively only in the fact that the latter possess α, β unsaturation in ring I. Kendall et al. (26) state that: "If the double bond 4, 5 is reduced with the rest of the molecule unchanged, physiologic activity is destroyed." Our assay results are not in agreement

with this observation since we find that our substance which can be represented by formula IV assays approximately 3 to 4 R.U. per mg., whereas our substance represented by formula II assays slightly less than 2 R.U. per mg. However, in agreement with Kendall's statement, we find that our substance represented by formula V is practically inactive as compared to our substance represented by formula I which assays approximately 1 R.U. per mg.



I. Compound *E* of Kendall, Substance *F* of Wintersteiner and Pfiffner, Substance *Fa* of Reichstein.
II. Substance M of Reichstein.
III. Compound A of Kendall, "Dehydro-corticosterone."
IV. $C_{21}H_{32}O_5$
V. $C_{21}H_{32}O_6$

Steiger and Reichstein (19) have recently reported the synthesis of the α,β-unsaturated ketone Δ^4-pregnen-diol 20, 21, one 3, which does not possess the α-ketol side chain and differs from Reichstein's substance *E* only in that the latter possesses hydroxyl groups in positions 11 and 17. This substance they found to be inactive by the Everse-DeFremery test at a level of 2 mg. per rat per day. Substance C of Reichstein (our substance represented by formula IV), which possesses the α-ketol side chain, but no α,β-unsaturated ketone group, has been found by us to assay approximately 3 to 4 R.U. per mg. The possession therefore of the α-ketol side chain seems more important for biological activity than the possession of an α,β-unsaturated ketone group.

Although some of these substances, especially those represented by formulas I, II, III and IV, apparently possess cortical hormone activity as evidenced by maintenance of life and growth in completely adrenalectomized rats, none of them are as active as either the crude starting material used for the fractionations reported herein, which assayed 4.5 R.U. per mg., or certain amorphous fractions which assayed 20 to 30 R.U. per mg. Thus, our results are in agreement with the findings of Wintersteiner, Reichstein, and Kendall, in demonstrating that the crystalline compounds which have thus

far been isolated analytically pure from adrenal extracts are relatively much less active than certain amorphous fractions from which they have been separated.

SUMMARY

Adrenal cortex extract prepared by our previously reported process has been purified in an ethyl acetate solution by removal of acidic and basic substances, followed by distribution between benzene and water, and finally by fractionation with Girard's reagent *T*. This distribution of cortical hormone activity has been quantitatively followed by our previously described rat method.

Six crystalline substances have been isolated, 5 of which we believe identical with substances previously reported by Kendall et al., Wintersteiner and Pfiffner, or Reichstein. The other crystalline substance apparently differs from any previously reported.

Substances *E* of Kendall and *M* of Reichstein, assayed 1 to 2 R.U. per mg.; *A* of Kendall ('dehydro-cortico-sterone' of Reichstein) approximately 3 to 4 R.U. per mg.; the substance, believed to be identical with *C* of Reichstein, to which biological activity had not previously been ascribed, approximately 3 to 4 R.U. per mg. Substance *D* of Reichstein and an unidentified substance of melting point 231–234° were found to be practically inactive when assayed at a level of 2 R.U. per mg.

The most active of the crystalline substances defined assay approximately 3 to 4 R.U. per mg. as compared to 20 to 30 R.U. per mg. for certain amorphous fractions obtained.

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ELECTROCARDIOGRAPHIC CHANGES DURING HYPOGLYCEMIA AND ANOXEMIA

CORTICAL DEPRESSION AND AUTONOMIC RELEASE

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DURING THE insulin and metrazol treatments of schizophrenia, evidences of overactivity of the autonomic nervous system have been observed, that is, changes in heart rate, salivation and perspiration (1). It is probable that these treatments affect the metabolism of the brain (2). Since autonomic centers are located in the brain, it becomes important to correlate the peripheral function of the autonomic nervous system with cerebral metabolism. Cardiac activity accurately reflects changes in the autonomic nervous system which can be easily detected by the use of the electrocardiograph. For this reason, electrocardiographic changes were correlated with those of cerebral metabolism during hypoglycemia and acute anoxemia induced by various agents.

METHODS

Hypoglycemia was produced in 5 dogs in post-absorptive condition by injection of protamine zinc insulin the night before the experiment and standard insulin on the following day in doses adequate to produce coma and marked slowing of the heart rate. The therapeutic effect of intravenous injections of glucose in these dogs was also observed. Anoxemia without accumulation of CO_2 (3) was induced in 9 dogs by inhalation of nitrous oxide or nitrogen. Intravenous metrazol injections were also given to 3 dogs since it has been previously observed that convulsions induce anoxemia (4). These agents were also administered in 4 hypoglycemic dogs. In all cases, anoxemia was continued until cardiac changes were observed and then oxygen administered. These changes were recorded continuously for periods of 2 to 7 minutes using lead II of the electrocardiograph. During this interval, samples of venous blood from the superior longitudinal sinus and arterial blood were drawn and analyzed for glucose (5) and oxygen (6).

Sixteen experiments were performed in all. In 9 of these electrocardiographic disturbances during hypoglycemia and anoxemia were observed before and after bilateral vagotomy. In 2 dogs, a complete stellate ganglionectomy and splanchnectomy were performed before the above procedures were done. These studies were carried out in 11 cases with intraperitoneal injection of sodium pentobarbital and, to simulate the treatment of schizophrenia, in 5 instances without any premedication.

RESULTS

Table 1 represents typical results of simultaneous observations on blood sugar and oxygen as well as on the electrocardiogram during short bouts of hypoglycemia or anoxemia. The electrocardiographic results may be divided in two groups: *a*), the effect on the autonomic nervous system and *b*), the direct effect on the heart.

Dogs with vagi intact during acute hypoglycemia, or anoxemia induced by various agents, exhibited a picture of widespread autonomic stimulation with an initial transient prepotence of the sympathetic and later predominance of the parasympathetic branch. In practically all instances and particularly in the anoxemia experiments a brief regular tachycardia of S-A origin with shortening of the R-T interval and T-wave changes were first observed. However, as soon as the blood sugar levels and A-V oxygen differences were very low (range 13–25 mg. per cent and 0.17–4.3 volumes per cent respectively), effects stimulating vagal influence were obtained on the electrocardiogram. These vagal cardiac effects varied somewhat but in hypoglycemia experiments consisted chiefly of: progressive slowing of the heart, bradycardia, and one instance of P-R prolongation, and partial heart block. The early T-wave changes in height and width were augmented and there were few auricular and more frequently ventricular extra-systoles. In anoxemia experiments, in addition to all these changes, there was frequent loss of S-A rhythm and occasional A-V dissociation.

After section of the vagi, in all observations no parasympathetic influence on the cardiac mechanism was noted during hypoglycemia or acute anoxemia. Instead, the normal control electrocardiogram showed a great increase in heart rate until a marked sinus tachycardia resulted. This continued for an appreciable length of time and until the premortal direct local effect of hypoglycemia and anoxemia were observed. These results following vagal section suggested that during marked hypoglycemia or anoxemia, the primary influence on the cardiac mechanism is of parasympathetic origin.

The direct local cardiac effects in marked hypoglycemia or anoxemia were indicated by inversion and changes in height and width of the T-waves as well as by a variable decrease in the height of the R-complex. S-T segment deviations were always found in anoxemia experiments and only once during hypoglycemia. After section of the vagi, the same local changes were noted for a long time but as the premortal stage was approached, the marked anoxemia depressed the S-A rhythm and A-V conduction to a great extent. Finally, ventricular fibrillation supervened.

In two experiments a bilateral stellate ganglionectomy and splanchnic resection were performed to remove sympathetic influence on the heart. The changes observed before and after sectioning of the vagi and during the anoxemia following nitrous oxide administration were essentially similar to those found with the sympathetic nerves intact.

The electrocardiographic changes during acute anoxemia varied in time of appearance as well as in character with the different agents employed. Significant disturbances were generally noted within 2 to 3 minutes after

onset of anoxemia with nitrous oxide, 1 to 3 minutes after the intravenous injection of metrazol and 6 to 7 minutes after the beginning of nitrogen administration. In all instances, the most marked cardiac disturbances were found when nitrous oxide was given, probably due to central anoxia augmented by the intrinsic narcotic effect of the drug. Electrocardiogram changes following metrazol injections (5 to 12 cc.) were temporary, appearing 30 to 50 seconds after the end of a severe convulsion and lasting only $\frac{1}{2}$ to 2 minutes.

The preparation of hypoglycemic and anoxemic dogs was performed with

TABLE 1

Agent used	Blood sugar mg. %	A:V diff. O_2 vol. %	Electrocardiogram	Remarks
Insulin	77	7.00	Sinus tachycardia, rate 215/m.	*Control* ecg, Zinc protamine given night before. Surgery under Nembutal anesthesia
	25	4.3	Rate 50/m., extra-ventricular systoles, T_2 absent S-T normal, (coupled beats).	After 80 U of standard insulin.
	83	7.17	Sinus tachycardia, rate 200–250/m.	20 gm. glucose intravenous before ecg.
	33		Sinus tachycardia, rate 300/m. T_2 inverted.	110 min. after vagal section and 60 U of insulin.
Nitrous oxide	61	5.45	Mild sinus tachycardia, rate 158/m.	*Control* ecg, surgery after nembutal anesthesia.
	62	0.17	Progressive P-R prolongation—heart block—A-V dissociation, rate 53—33, R small, S-T depressed, occasional extra-ventricular systoles.	$2\frac{1}{4}$ min. after N_2O.
			Ecg, essentially normal, rate 91/m.	After O_2.
	93	0.28	Regular sinus tachycardia, rate 188/m. S-T_2 elevation, T_2 wide and high.	Vagi sectioned, ecg taken $3\frac{1}{4}$ min. after 2nd bout of N_2O.
			Sinus tachycardia, rate 160/m. S-T_2 and T_2 normal.	After O_2.
Nitrogen gen	126		Mild sinus tachycardia, rate 150/m. T_2 inverted.	*Control* ecg, nembutal anesthesia.
	138	0.79	Progressive decrease in heart rate 150—94—70/m. P-R normal, S-T_2, elevated, T_2 tall, wide.	7 min. after N_2.
			Control ecg, rate 167/m.	After O_2.
	119	0.72	Marked sinus tachycardia, rate 245/m. T-P_2 fusion, R_2 smaller, S-T_2 elevated.	After vagal section and $4\frac{3}{4}$ min. after 2nd bout of N_2.
			Control ecg, rate 214/m., T_2 diphasic.	After O_2.

TABLE 1.—*Continued*

Agent used	Blood sugar mg. %	A:V diff. O_2 vol. %	Electrocardiogram	Remarks
Metrazol	93		Sinus tachycardia, rate 200/m.	*Control* ecg, nembutal anesthesia.
	98		Progressive decrease in heart rate. 200–150–120/m., mild S-T_2 elevation.	1½–2 min. after intravenous injection 8 cc. metrazol.
			Control ecg, rate 188/m.	½–2 min. after last convulsion.
		O_2 Content 22.66	Sinus arrhythmia, normal ecg, rate 137/m.	*Control* ecg, *no* premedication except local anesthetic for surgery.
		4.39	Rate, 88–100/m., irregular rhythm, extra-auricular systoles, S-T normal.	Control ecg taken first. 2 min. after intravenous injection of 5cc. metrazol after convulsion.
			Bradycardia 58/m., P-R prolongation, S-T_2 depression, T_2 diphasic.	6 min. later after second series of convulsions.
			Bradycardia 48/m., P-R_2 prolongation, heart block, S-T_2=normal.	9 min. later after third series o mild convulsions.
		24.47	Same as control, rate 136/m.	15 min. later, no convulsions for 6 min.

and without the use of sodium pentobarbital anesthesia. The results in either case were essentially the same. However, it must be stated that premedicated dogs generally showed minor T-wave changes and a moderate sinus tachydardia before insulin or anoxemic agents were employed. Further, anoxia was more easily produced by the agents used in the already depressed animal.

All electrocardiographic changes of central or local origin during hypoglycemia or anoxemia obtained before or after vagal section promptly disappeared after the administration of intravenous glucose or intratracheal oxygen respectively. Control electrocardiograms were obtained within 1 to 3 minutes after the blood sugar levels and A:V oxygen differences were restored to normal. No permanent functional change, as determined by the electrocardiogram was found after the short interval of the marked hypoglycemia or anoxemia.

DISCUSSION

With the aid of blood sugar and oxygen determinations and the use of the electrocardiogram as an indicator, it was possible to study the effect of marked hypoglycemia and anoxemia on the autonomic nervous system. In addition to the T-wave changes of the electrocardiogram of hypoglycemic dogs previously reported (7, 8, 9) a definite but delayed parasympathetic influence shown by the slowing of the heart and bradycardia was detected. Such an effect was observed only when blood sugar values were within the range of 13 to 25 mg. per cent and explains probably why it was not noted by previous workers. That this influence is of parasympathetic nature is

indicated by its absence after bilateral vagotomy and its continuance after stellate ganglionectomy and splanchnic nerve resection.

The electrocardiographic changes noted during anoxemia induced by different procedures confirm in all respects those reported by the thorough investigation of Greene and Gilbert (10). As previously expressed (11, 12) the effect on the heart noted during anoxemia is that of vagospasm. However, by the determination of blood oxygen and A-V differences in our experiments, we have been able to study the threshold levels at which oxygen deprivation stimulated autonomic activity. Such a correlation has not been made previously. It is significant therefore that autonomic influence becomes manifest during hypoglycemia whenever the oxygen A:V difference is less than 4.3 volumes per cent (in our dogs). During anoxemia these differences were much smaller though only for a short period. In the light of energy demands of brain metabolism as a whole, it is of considerable interest to note the anoxic as well as the hypoglycemic levels at which parasympathetic nervous system activity is augmented.

From the data presented it may be seen that hypoglycemia and anoxia exert similar effects on the autonomic nervous system. The initial cardiac changes during both hypoglycemia and anoxia were transient and of sympathetic origin and soon gave way to parasympathetic predominance. Cannon, McIver and Bliss (13) have previously shown that the sympathetic effects occur with low blood sugar. However, in more marked hypoglycemia such as noted in the present experiment, when vagi are intact, the cardiac mechanism is predominantly under parasympathetic influence. With anoxia, the same sequence of events has been noted, that is, an initial sinus tachycardia due to sympathetic influence followed by the predominating cardiac change of parasympathetic origin.

It should be emphasized that both hypoglycemia and anoxia are equally capable of diminishing metabolism of the brain. Therefore it might be expected that these procedures would induce similar changes. Gellhorn (14) has repeatedly emphasized the synergistic action of hypoglycemia and anoxemia particularly on the function of the sympathetic nervous system. The mechanisms, however, by which this action is produced, are entirely different. Nitrogen, nitrous oxide, and metrazol all reduced the oxygen available to the entire organism including the brain. Insulin, on the other hand, causes the effects by depriving the brain of its chief foodstuff, carbohydrate. No changes of the autonomic nervous system were observed when the oxygen A-V differences were within normal limits. With decreased metabolic rate, depressed cerebral function might be expected. This depression would manifest itself first in the parts of the brain with greatest metabolic activity. As Heymans et al. (15) and others have shown, the cortex is one of these parts. Hughlings Jackson, in expressing his concept of the cephalad dominance of the central nervous system, stressed cortical inhibition of the functions of the lower parts of the brain. With depression of the cortex, therefore, loss of cerebral inhibition and release of sub-cortical functions might be expected. This release is expressed among other ways by increased activity

of the autonomic nervous system, an increase probably mediated by centers situated in the hypothalamus and medulla oblongata.

SUMMARY

The activity of the autonomic nervous system of dogs was studied during hypoglycemia and anoxemia. Electrocardiographic changes showed that in both conditions, an initial transitory sympathetic influence was followed by parasympathetic predominance. This increased activity of the autonomic nervous system was associated with a depressed metabolism of the brain as indicated by diminished cerebral utilization of oxygen. It is concluded that with depression of the brain metabolism, the predominant and ultimate effect on the autonomic nervous system is a release of parasympathetic influence.

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HYPERGLYCEMIA WITHOUT GLYCOSURIA ASSOCIATED WITH DISTURBANCES IN METABOLIC PROCESSES

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UNDER THE TITLE "Hyperglycemia Without Glycosuria" a brief report of cases encountered in 1936 and 1937 appeared in this Journal in April, 1938. The purpose of the present report is to point out the association between hyperglycemia and deviations in a wide variety of metabolic processes and to advance the theory that hyperglycemia, per se, may be one of the determining factors in certain clinical manifestations.

Hyperglycemia has generally been thought of as a symptom, an effect in the course of diabetes mellitus. It has not generally been considered as a causative factor responsible for disturbances in metabolism which might be reflected in clinical manifestations. The demonstration of hyperglycemia without glycosuria in a large number of patients presenting a wide variety of complaints has given us an opportunity to observe the effects of treatment designed to correct this deviation in metabolism. Improvement in clinical manifestations associated with the use of such therapeutic measures has occasioned the development of a theory that hyperglycemia may act as a determining factor in initiating and in maintaining unfavorable metabolic conditions which, in turn are reflected clinically.

The following case histories, deleted of irrelevant material, are recited as illustrations of instances in which clinical improvement occurred with the application of measures designed to correct hyperglycemia, the existence of which has been demonstrated through glucose tolerance testing.

CASE REPORTS

Case 1. R.W., male, 8 years old, was admitted to the Orthopedic Hospital service Oct. 22, 1921 on account of a green stick fracture of the right ulna and radius. He was readmitted July 22, 1924 on account of flat feet. From this date until Jan. 19, 1928 he was kept under more or less continuous observation. Wedges in the heels and soles, stretching of muscles and tendons and plaster casts were resorted to without lasting effect. A note dated July 1, 1927, made the first mention of difficulty experienced in climbing stairs and described muscle weakness—in abdominal muscles, about hips, especially the flexors, and in both flexors and extensors about the knees. The possibility of pseudohypertrophic muscular dystrophy appears in the note of this date. Stretching of the heel cords was advised.

During the next 4 months the patient was taken to one private physician who removed his tonsils and to another for study and diagnosis. The study resulted in a diagnosis of pseudo-hypertrophic muscular dystrophy. The next visit to the Ortho-

pedic Hospital, Dec. 10, 1927, was on account of a fractured humerus sustained in a fall. A note dated Dec. 27, 1927, states, "Condition of dystrophy becoming more marked—cannot walk up hills or climb stairs."

He was referred for an endocrinological opinion. The muscular weakness, by Jan. 15, 1928, had progressed until the child was unable to turn when in a prone position. He was unable to rise from a prone to a sitting position. A glucose tolerance test on Jan. 26, 1928, showed hyperglycemia without glycosuria. It showed a fasting blood sugar level of 136 mg. per cent; ½ hour after the ingestion of 100 gm. of glucose 187 mg.; 1 hour later 210 mg., 2 hours later 166 mg.; and 3 hours later 150 mg.

On a diabetic diet[1] improvement was almost immediate. Weekly observation recorded continuous favorable progress. A note dated April 17, 1928, states, "Patient is able now, with some effort, to rise from the ground without any assistance and without grasping any objects." A note dated Aug. 14, 1928, states, "He can rise without putting his hands to his knees." A note dated April 2, 1929, states, "On examination muscle power cannot be distinguished from normal." A note dated May 28, 1929, states that, "He is delivering 88 papers daily and 40 Sunday papers." He had improved until he was able to carry on as a perfectly normal boy. During close observation throughout the next 2 years he twice reported a sense of decreasing strength in the muscles of his back, especially in the lumbar region. Each time he admitted having varied his diet by going back to taking freely of concentrated carbohydrates, especially of bread. Correction of this deviation from the prescribed diet each time was followed by a cessation of the sense of decreased muscle power in the back. His complete recovery persisted, and for years he has worked as a regular employee at a department store in Seattle.

Case 2. Mrs. W., aged 45, was seen in 1928 on account of distress and disability incident to lack of healing in a surgical wound incurred about six months before in an attempt to produce a stiff joint in a flail ankle. The case had presented no unusual difficulty nor occasion for concern, but the operative wound had suppurated and had resisted all attempts to promote healing. The condition changed during several months from bad to worse, with increasing physical distress, constant pain, malnourishment, and associated mental anxiety. There had been a loss of 30 lb. in weight. A glucose tolerance test showed hyperglycemia without glycosuria, and gave blood sugar values as follows: fasting, 135 mg. per cent; ½ hour after the ingestion of 100 gm. of glucose, 240 mg.; 1 hour later, 200 mg.; 2 hours later, 180 mg.

The patient was placed on a diabetic diet regime. Without other change in care or medication the response was prompt and gratifying. Within a week the pain began to abate, the appetite improved, restfulness and sleep became the rule rather than the exception. Within a month the patient, who had been strictly confined to bed and uncomfortable on an air mattress, was able to sit up in a chair. Within 3 months she was able to begin to resume her duties about her home.

The subsequent history in this case is pertinent. In June, 1936, she suffered an attack of pain in the abdomen and exhibited a typical picture of an infected gallbladder. Palliative measures for 36 hours gave no relief. With symptoms growing more pronounced an immediate operation was decided upon. On opening the peritoneal cavity free blood-stained fluid was found; areas of fat necrosis were seen. The gallbladder, containing one large stone, was found to be distended and was removed. Deep exposure, in the location of the head of the pancreas, revealed an edematous,

[1] The terms, 'diabetic diet' and 'diabetic dietary regime,' are used to signify a dietary regime in which carbohydrate intake is restricted almost completely to foods of low carbohydrate content, and the total carbohydrate intake is restricted always to less than 150 gm.

hemorrhagic mass flecked with areas of fat necrosis. The patient made a satisfactory recovery. A glucose tolerance test in December 1936 gave a normal blood sugar curve.

Case 3.[2] *J.F.*, a male, was 74 years old, single. On Sept. 12, 1935 he had a transurethral resection which failed to enable him to empty the bladder. On Sept. 30, 1935 the operation was repeated. On Oct. 9, 1935, suprapubic prostatectomy was done because the patient still was unable to empty his bladder and the prostate was large and soft. The wound suppurated and refused to heal. On April 6, 1936, he was discharged from the hospital. Through months there was no progress toward healing. On Sept. 16, 1936, he was readmitted to the Swedish Hospital and a plastic repair was made on the suprapubic sinus; the wound edges were brought together. The whole wound promptly broke down. After a month's time the wound had shown little or no tendency to heal even with the aid of heliotherapy and the application of various medicaments. A glucose tolerance test showed hyperglycemia without glycosuria. It showed a fasting blood sugar level of 133 mg.; ½ hour after ingestion of 100 gm. of glucose, 200 mg.; 1 hour after, 222 mg.; 2 hours after, 222 mg.; and 3 hours after, 133 mg.

He was then put on 15 U of insulin t.i.d. and a diabetic diet. Without other therapy, and only dry gauze on the wound, healing began and within 10 days the sinus was closed. It has remained closed.

Case 4. B.P., a female, 12 years of age was admitted June 1, 1936. Her complaint was rheumatic pains for the past month, especially in the site of a left ankle fracture incurred 5 years previously. Examination on admission revealed nothing pertinent.

A note by the medical service, June 9, 1936, commented on her overweight. She was 65 in. tall, and weighed 142 lb. Besides recording the complaint of pain in her foot, ankle, leg, and arm, it was noted that when stooping she felt as if her eyes were about to fall out. It was also noted that pain in the left ankle had been more or less continuous since the accident. She was referred to the arthritis clinic. From the arthritic clinic she was referred for endocrinological study. A note on June 16, 1936, recorded that she had begun to menstruate a year previously. The periods were reported as regular and without pain. School progress had been fairly satisfactory. Food consisted largely of articles of high caloric content. Glucose tolerance test showed a fasting blood sugar of 161 mg.; ½ hour after ingestion of 100 gm. glucose, 217 mg.; 1 hour after, 178 mg.; and 2 hours after, 156 mg.

On a diabetic dietary regime all the pain of which she complained subsided promptly. Pain in the ankle which had been of sufficient intensity to interfere seriously with school attendance ceased and the ankle gave no further trouble.

Case 5. V.B., a female, aged 12, was admitted Jan. 13, 1936, complaining of aching in the upper portion of her back. The admission note states that examination showed definite tenderness to deep pressure over the dorsal spine and considerable muscle spasm over the lumbar spine. X-ray of the spine was interpreted as showing an extreme degree of epiphysitis involving the lumbar and dorsal spine. She was admitted for frame treatment because of her painful back, which was noted to have had its onset with a fall about 2 months previously.

She was discharged, Feb. 9, 1936, wearing a back brace. Because of overweight, a diet low in caloric content was advised. Progress was not satisfactory and she was re-admitted on May 16, 1936, and remained in the hospital for physiotherapy, postural training, and rest on a frame. She was discharged Sept. 9, 1936, wearing a brace. Diagnosis a second time was of epiphysitis of lumbar spine. Progress was not

[2] History kindly furnished by Dr. A. H. Peacock.

satisfactory. A month later she was referred for a metabolic study. A glucose tolerance test showed a fasting blood sugar of 182 mg.; ½ hour after ingestion of 100 gm. of glucose, 200 mg.; 1 hour after 192 mg.; and 2 hours after, 166 mg., with no glycosuria. On a diabetic dietary regime the discomfort subsided promptly.

A note by the orthopedic service dated Jan. 5, 1937, stated that discomfort and tenderness in spine had ceased and the use of the back brace was discontinued. There was no recurrence of the discomfort.

Case 6. Miss N., private case referred by Dr. John Lecocq, age 29, was first seen Sept. 25, 1936. She complained of gaining weight and backache. Pain that began 6 weeks previously had become almost incapacitating during the past 3 weeks. Orthopedic measures had failed to give relief. Examination showed a moderately obese woman, (obesity of pituitary type) with tenderness to pressure in the lower lumbar region. History and examination were otherwise essentially non-informative. A glucose tolerance test revealed hyperglycemia without glycosuria. It showed a fasting blood sugar of 164 mg.; ½ hour after ingestion of 100 gm. of glucose, 234 mg.; 1 hour after, 197 mg.; and 2 hours after, 208 mg. A diabetic dietary regime, low caloric content food, and ½ grain of thyroid once a day, without any other therapeutic measures gave complete relief within 2 weeks. There was no recurrence during the ensuing year.

Case 7. D. C., seen in the Providence Hospital in consultation with Dr. J. Irving Tuell, was a boy 13 years old, hospitalized on account of a slipped epiphysis. This child presented a typical picture of Fröhlich's syndrome with marked obesity and lack of development of the penis. A glucose tolerance test showed a fasting blood sugar level of 141 mg.; ½ hour after ingestion of 100 gm. of glucose, 300 mg., 1 hour after, 250 mg.; 2 hours after, 214 mg.; and 3 hours after, 80 mg. He was put on a diabetic regime, low caloric content diet to favor reduction, and antuitrin-S. Progress was very satisfactory.

Case 8. Mrs. J. McN., aged 66 years, was seen in the Providence Hospital in consultation with Dr. James Bowers. Evidence of occlusion of cerebral vessels had developed. The onset of her disturbance and the progress were atypical. A fasting blood sugar test had been made and the result found to be 124 mg. per cent. A glucose tolerance test showed hyperglycemia without glycosuria. It showed a fasting blood sugar of 118 mg.; ½ hour after ingestion of 100 gm. of glucose 206 mg.; 1 hour after, 260 mg.; 2 hours after, 276 mg.; and 3 hours after 272 mg. With the use of insulin and a diabetic diet this patient showed definite improvement by promptly recovering from a semicomatose condition. Although hemiplegia was not altered, she left the hospital in fair condition and continued so for several weeks.

Case 9. Mrs. W., seen in August, 1937, was a housewife 70 years old. She complained of gradually increasing disability incident to rheumatic pains. Her left knee had become swollen and painful, and exhibited a moderate amount of effusion. A glucose tolerance test showed a fasting blood sugar of 170 mg.; ½ hour after ingestion of glucose, 200 mg.; 1 hour after, 189 mg., 2 hours after, 217 mg.; 3 hours after, 125 mg.; and 4 hours after, 108 mg. There was no glycosuria. A diabetic regime without other therapeutic measures was followed by decreasing pain and disability. The knee, which for months had progressively grown worse, became less annoying. Within 6 months it had returned to normal dimensions and became free from pain.

Case 10. Mrs. E. K., age 58 years, presented the complaint of ulceration involving both legs from the ankles half way to the knees. The lesions beginning about 7 weeks previously had gradually grown worse. Various kinds of treatment had been tried with no permanent benefit. A glucose tolerance test showed hyperglycemia

without glycosuria. The fasting blood sugar was 147 mg. per cent; ½ hour after ingestion of glucose it was 192 mg.; 1 hour after 200 mg.; 2 hours after 277, three hours after 200, and four hours after 112.4. The use of a diabetic diet regime without insulin gave some improvement. The use of insulin 10 U t.i.d. was followed by almost complete amelioration of pain and evidence of a tendency towards more rapid healing. Within 2 months the deeply ulcerated areas were restored to normal and covered with new epithelium.

Case 11. Miss B.C., age 25, was seen Sept. 1, 1937, on account of undue fatigue. During the summer session as a student in the university she found it necessary to lie down at least 2 hours every afternoon even with retiring at 10 and not rising until 8 A.M. Complete laboratory study showed hyperglycemia without glycosuria. Fasting blood sugar was 133 mg. per cent; ½ hour after ingestion of glucose 204, 1 hour after 200, 2 hours after 149, 3 hours after 133, and 4 hours after 98. A diabetic diet regime gave prompt restoration to average endurance and rest periods during the day were dispensed with.

Case 12. Miss G.L., age 39, was seen Aug. 27, 1937. She complained of lack of endurance and emotional instability. She was terrified with the idea of trying to do a certain phase of the work in her course as a university graduate student. Complete study showed: hemoglobin 80 per cent; red blood count 3,380,000; B.M.R.—21; glucose tolerance test, fasting blood sugar 163.9 mg., ½ hour after ingestion of glucose 208, 1 hour after 138, 2 hours after 156, 3 hours after 131, and 4 hours after 122 mg. per cent. The use of lextron and thyroid, ½ grain daily, was followed by an unsatisfactory degree of improvement. The use of insulin 10 U t.i.d. and a diabetic dietary regime was followed by complete recovery within 2 months.

Case 13. Mrs. J.L., was seen for minor complaints through 1934–35 and 36. She complained on Aug. 19, 1936, of pain in left side of her face as if the antrum were involved. An examination showed that the antrum was not infected. The pain in the left side of the face gradually subsided but discomfort developed in the region of the right masseter muscle. Apparent atrophy in this region complicated the picture. This region was seldom free from a sensation of tension and stiffness often so intense as to give actual pain. No possible focus of infection could be demonstrated with the exception of the tonsils. These were removed on Aug. 28, 1937. There was no change in the condition in the right side of her face. Vitamin B gave no relief. Sedatives and soporifics gave no lasting relief.

A complete laboratory study showed hyperglycemia without glycosuria. Fasting blood sugar was 149 mg., ½ hour after ingestion of glucose 156 mg., 1 hour after 200 mg., 2 hours after 128 mg., 3 hours after 125 mg., and 4 hours after 111 mg.

A diabetic dietary regime was of no avail. The administration of 5 U of insulin t.i.d. gave more relief than had been obtained from any previous medication. The decrease in the annoyance was sufficient to enable the patient to get a moderate amount of sleep which had not been possible for months. Increase in the dose of insulin to 10 U was accompanied with complete abatement of the distressingly painful sensations. Continuance on that dose of insulin t.i.d. with a diabetic dietary regime has been associated with a continued freedom from the annoying sumptoms except the apparent atrophy. The area over the right masseter still appears atrophic, though the masseter muscle has never seemed to be involved.

The question of the relationship between hyperglycemia without glycosuria and hyperglycemia with glycosuria is suggested by the familial incidence in the following 4 cases. Clinical cases other than these four have not offered opportunity for investigating the possibility of familial influence.

Case 14. Miss A.B., was seen June 21, 1935, having experienced some sort of a convulsive seizure the preceding afternoon, which had left her with a hemiplegia. The patient was 55 years old. She had had medical care rather infrequently during the preceding 7 years, including radium to a fibroid uterus and a tonsillectomy. Results of a physical examination were essentially negative. A glucose tolerance test showed hyperglycemia practically without glycosuria. Her fasting blood sugar was 149 mg., ½ hour after ingestion of 100 mg. of glucose 233 mg., 1 hour after 250 mg., 2 hours after 151 mg., 3 hours after 175 mg., and 4 hours after 104 mg. The urine showed a trace of sugar at the end of 3 hours. It is interesting to note that with the drop of blood sugar from 175 to 104 mg., there developed in her left hand sensations comparable to those with which her seizure had been initiated the preceding afternoon. Examination at this time showed a decreased kinesthetic sense in the left hand and a decreased muscular control. With the administration of a glass of orange juice the disturbance in sensation disappeared within the next 10 minutes.

The patient was placed on a diabetic dietary regime. Within 2 weeks she had completely recovered to her former state of health. She returned to her home where she carried on her work as a teacher for the next year, during which time she adhered only under compulsion to the dietary restrictions which she felt had been imposed. Subsequently she refused to observe her dietary restrictions. Her subsequent history reports the development of diabetes, hemiplegia and death.

Case 15. A sister, Miss M. B., age 45, was seen Jan. 7, 1938, with a complaint of weakness, asserting she was unable to carry on with her work as a teacher of singing. Her family history was non-informative except for the previous record concerning her sister. Her personal history included a laparotomy for gallbladder and appendix in 1932, intrauterine polyps in 1923, and radium for uterine hemorrhage in 1936. Physical examination gave essentially negative findings. A complete laboratory test showed hyperglycemia without glycosuria. Fasting blood sugar was 152 mg., ½ hour after ingestion of glucose 189 mg., 1 hour after 200 mg., 2 hours after 104 mg., 3 hours after 130 mg., and 4 hours after 110 mg.

She was placed on a diabetic dietary regime and received theelin as treatment for hot flashes, 1 cc. twice a week, for the next 6 weeks. Laboratory tests had also showed hemoglobin 70 per cent, red blood count 3,330,000. She received lextron, 1 capsule t.i.d., for the anemia. She noted improvement from the beginning and at the end of 2 months felt completely recovered.

Cases 16 and 17. The other two cases are those of D.S. and N.S., identical twins, 11 years of age. One of these twins gave a history of having suffered difficulty in the form of a nervous compulsion. This nervousness was expressed especially in the form of anxiety concerning her mother. She would leave her place in the classroom in school and rush home to greet her mother with the inquiry, "Are you all right?" If questioned why she had come home she would say that she was anxious lest something might have happened to her mother. If the mother went out and left the child at home, the child was in distress until the mother should return. A psychiatrist had been consulted and pronounced the case one of a 'spoiled child.' A psychologist was then consulted who pronounced the case one of nervous compulsion. The children were referred for study.

The results of a physical examination were essentially negative except for possible anemia and moderate fatigue posture and slight undernutrition. The laboratory findings were within average limits for both except for anemia and deviations in glucose tolerance tests. The child that complained of the nervous compulsion showed hemoglobin 75 per cent, red blood count 3,650,000; the sister hemoglobin 70 per cent, red blood count 3,200,000. A glucose tolerance test on D.S. showed: fasting

blood sugar 172 mg., ½ hour after ingestion of glucose 277 mg., 1 hour after 222 mg., 2 hours after 187 mg., 3 hours after 151 mg., and 4 hours after 117 mg. A similar test on *N.S.* showed: fasting blood sugar 135 mg., ½ hour after ingestion of glucose 250 mg., 1 hour after 186 mg., 2 hours after 163 mg., 3 hours after 107 mg., and 4 hours after 96 mg. Both children were placed on a liver and iron preparation, on a diabetic dietary regime, increased caloric intake, and increased rest. Without other suggestions or other medication, the nervous compulsion behavior had largely ceased to be a problem within one month.

DISCUSSION

That the above reported cases, except *14* could not be considered typical diabetics in the sense that they exhibited hyperglycemia with glycosuria, is attested by the fact that repeated urine examinations failed ever to reveal sugar in the urine. The cases longest under observation, the first and second in the list, had urine examination at various times and under varying conditions throughout years of care. The third in the list, a urological case, had spent months in a standardized hospital during which time there were repeated urine examinations each of which recorded no evidence of sugar in the urine. Every other case here reported had repeated urine examinations without ever finding sugar in the urine, even with repeated glucose tolerance tests. The fourth and the seventh in the list are illustrative of the intensity of study to which cases have frequently been subjected and the care exercised in testing for glycosuria.

Case *4* had glucose tolerance test findings as follows

6– 6–36	161	217	178	156	
10–30–36	176	272	166	187	167
1–15–37	145	217	148	153	168
8–24–38	121	143	98	102	89

with urine specimens obtained at the time of taking each blood specimen. No specimen of urine ever showed even a trace of sugar.

Case *6* in the series had glucose tolerance test findings as follows:

10–31–36	141	300	250	214	80
11–23–36	100	142	180	136	116
11–30–36	104	217	206	130	108
12– 9–36	106	106	996	86	85
12–21–36	108	130	112	105	100

with a specimen of urine obtained at the time of taking each blood specimen. As in the case just recited no one of these specimens of urine ever showed a trace of sugar, nor did any other specimen of urine tested during a 4-week sojourn in the hospital.

The question of import in all these cases is—does the hyperglycemia, per se, operate to adversely influence the metabolic processes involved or are the disturbances referable to the influence of one or more of the factors back of the hyperglycemia? This is a very pertinent question because we have been taught to think that the presence of an appropriate amount of insulin is

essential for the optimum utilization of carbohydrate. We have not so definitely been taught to think that the maintenance of an appropriate concentration of glucose in the blood stream is equally important. The explanations offered for the clinical manifestations commonly exhibited in association with hypoglycemia might be presented as a justification for the development of the theory that is being submitted. Hypoglycemia, just the opposite, in insulin and glucose contents, to hyperglycemia is prone to be associated with a wide diversity of clinical manifestations. The inappropriate concentration of glucose and not the inappropriate supply of insulin is ascribed as the direct cause back of such clinical exhibitions. Such histories as those submitted and experience with several score of other equally striking cases treated in the course of ten years of study and observation have prompted the formulation of the theory that hyperglycemia, per se, operates at times as a determining influence in the initiation and maintenance of metabolic disturbances which may be described as metabolic processes operating under other than optimum conditions and which in turn are reflected in clinical manifestations.

THE REGENERATION OF AUTOGRAFTS OF THYROID TISSUE IN PARTIALLY AND COMPLETELY THYROIDECTOMIZED RATS

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ON THE BASIS of experiments on the transplantation of parathyroid tissue, Halsted concluded that there must be a physiologic need for an endocrine principle in order that a graft of the gland elaborating that principle be successful. This 'law of deficiency' has been a subject of dispute in the literature on the transplantation of endocrine tissues. Loeb made the following statement.

> It is possible in inbred families to transplant successfully as many as four lobes of the thyroid gland, from one individual to another one, possessing its own thyroid gland, and thus to prove that a deficiency in the thyroid hormone is not required for the successful transplantation of this organ with internal reaction as had been assumed, among others, by Halsted.

In experiments by Higgins and one of us (Ingle) on the autoplastic transplantation of the adrenal glands in the rat, it was observed that a unilateral graft of an adrenal gland might remain viable in the presence of one intact adrenal but that little regeneration of cortex occurred unless a state of deficiency had been set up by removing both adrenal glands. Also Higgins and one of us (Ingle) have shown that the regeneration of the enucleated adrenal is completely suppressed in the presence of an excess of cortin. In the present study we have compared the regeneration of autografts of thyroid tissue in rats which had been partially thyroidectomized and in similar animals which had been completely thyroidectomized.

METHODS

The animals used in this experiment were sexually mature female rats of the Wistar strain having a range in body weight of 160 to 200 gm. Twenty-two animals were closely matched into pairs on a basis of body weight. Under aseptic conditions the thyroid was completely removed from one animal of each pair. Half of the gland was attached to the surface of the left ovary by means of a fine silk suture which passed through the body of the thyroid and around the body of the ovary. Hemorrhage from the ovary was successfully avoided. The other half of the thyroid was discarded. In the other animal of each pair, half of the thyroid was left in situ. The other half was removed and transplanted to the surface of the ovary. A pair of animals was killed at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12 weeks after operation. Four additional animals were completely thyroidectomized and one lobe was transplanted to the ovary. These animals were killed at intervals of 1, 2, 3, and 5 days after operation.

RESULTS

During the first 2 weeks after operation small losses in body weight were consistently observed among the completely thyroidectomized rats. The partially thyroidectomized rats did not lose in body weight during this period. These losses were regained within 3 weeks after operation and no consistent weight differences developed after this time.

At necropsy all of the thyroid tissues were fixed in formalin and embedded in paraffin and the entire blocks were cut into serial sections 8 μ in thickness. The sections were then stained with hematoxylin and eosin.

Sections of thyroid gland taken from several normal rats were used as controls. In every rat in which only half of the thyroid had been transplanted,

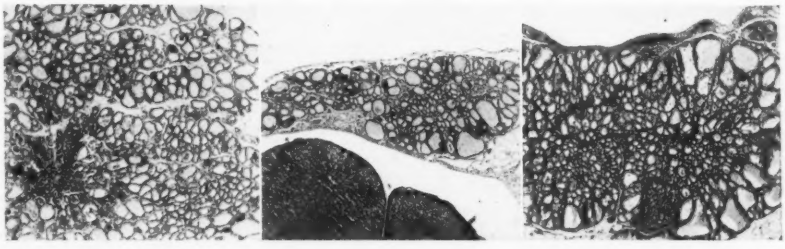

Fig. 1. NORMAL THYROID TISSUE LEFT IN SITU AFTER HALF OF THE GLAND HAD BEEN TRANSPLANTED (×24). Fig. 2. THYROID TISSUE REMOVED WITH OVARY FROM A RAT 12 WEEKS AFTER REMOVAL OF ONLY HALF OF THE GLAND. Note its similarity to the portion left in situ and its comparatively small size (×24). Fig. 3. THYROID TISSUE REMOVED WITH OVARY FROM A RAT 12 WEEKS AFTER COMPLETE THYROIDECTOMY. Its large size and signs of cellular hyperplasia can readily be seen (×24).

the thyroid tissue left in situ was found to be similar in every respect to the normal glands (fig. 1). The acini varied in size from small to moderately large and most of them contained considerable quantities of colloid which stained faintly pink with eosin. In no instance were any nodules or other signs of cellular hyperplasia observed. The cells lining the acini were of the low cuboidal type ordinarily seen in the normally functioning gland. They were arranged in even, single rows without papillary formation.

The thyroid tissue that had been transplanted to the ovary showed distinct differences in the completely thyroidectomized as compared with the partially thyroidectomized rats. Of the grafts removed 1, 2, 3, and 5 days after operation, those first removed showed a complete necrosis of almost all of the thyroid tissue except a few acini around the periphery where the blood capillaries had invaded the transplant early enough to keep them viable. In the succeeding specimens the necrotic tissue was seen to disappear gradually and to be replaced by fibrous connective tissue. By the fifth day the acini around the periphery began to increase in number so that by the seventh day they had practically doubled in the completely thyroidectomized animals. However, in the partially thyroidectomized animals, increase was so slight that in many instances it was difficult to ascertain definitely whether or not regeneration was actually occurring. The transplants removed two weeks after operation revealed definite evidence of regeneration in the animals which had been partially thyroidectomized. However, this fact was judged almost entirely by the increase in the total number of acini and not by

the cellular characteristics, as the new acini appeared to be morphologically similar to those of the normal glands. On the other hand, the thyroid tissue removed from animals 2 weeks after complete thyroidectomy showed a rapid and marked regeneration. Not only was there a great increase in the total number of acini but morphologic evidence of active cellular hyperplasia was observed. The new acini varied in size more widely than those in the grafts of the partially thyroidectomized animals and there was a diminution in the amount of interstitital connective tissue between them. The lining epithelial cells were more columnar in type and in many areas were packed in masses so that they appeared as double rows and even as small papillae. The cytoplasm was distinctly acidophilic and the nuclei were large and hyperchromatic. Colloid was seen in only a few of the larger acini and had a pale watery appearance.

This notable difference in morphologic characteristics between the grafts from the partially and from the completely thyroidectomized animals was clearly revealed in the transplants removed from the remaining experimental rats, which included all of the animals killed up through 12 weeks after the transplants had been made (fig. 2, 3). In spite of the signs of rapid regeneration in the transplants from completely thyroidectomized animals, there was a complete absence of nodular hyperplasia such as is commonly observed in the so-called adenomatous goiter in human beings.

An interesting corollary to our study of the thyroid was the presence, in many of the transplants, of apparently normal parathyroid glands. However, no special study of these organs was carried out.

COMMENT

Our studies tend to substantiate the view that the transplanted thyroid gland grows rapidly and shows microscopic evidence of increased cellular activity when a definite state of thyroid deficiency exists. However, when a portion of the thyroid gland is left in place, the transplanted portion grows very slowly without signs of cellular hyperactivity and never attains any appreciable size. This is in agreement with the results of the transplants of adrenal gland made by Higgins and one of us (Ingle) which have been previously mentioned.

The portion of thyroid gland left in situ after the other half had been transplanted might have been expected to show signs of increased growth and activity. Since it revealed no changes whatsoever, the supposition is that it was adequate to meet the demands placed on it. On the other hand, once a state of increased growth has been established in thyroid tissue, it apparently persists for a long period or at least the tissue retains the microscopic picture which suggests such a condition. We say this because, as late as three months after the transplant had been made in the completely thyroidectomized rats, the thyroid tissue attached to the ovaries retained the same characteristics of cellular hyperplasia and increased activity that it revealed 2 weeks after operation, at which time it was growing most actively.

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A COMPARISON OF THE DISTILLING AND DRY ASHING METHODS FOR THE DETERMINATION OF BLOOD IODINE

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THE ADVENT of blood iodine determinations as an adjunct to the clinician's and laboratory investigator's armamentarium has brought up the necessity of obtaining accuracy together with greater simplification in the methods employed. Whereas the earlier values of I_2 in blood were between 6 and 12 γ per 100 cc., with the refinement in methods and with an increased ability to secure practically iodine-free chemicals these values were gradually reduced so that roughly 2 to 4 γ is now being considered as the average normal amount of I_2 per 100 cc. of blood (1, 2). If blood iodine determinations are to become a routine laboratory procedure, a simplified and inexpensive method must of necessity be the one adopted. In order to establish that no sacrifice was being made to accuracy for the sake of simplicity, preliminary determinations were made on various samples of blood; first by Strickler's (2) modification of the McCullagh (3) dry ashing method, and then by Stevens' (4) distilling method as modified somewhat in this laboratory. After consistent results were obtained by both methods, a search of the literature failed to reveal any published reports comparing the blood iodine values by different methods as determined on the same sample of blood. We consequently decided to make such determinations in order to establish the comparative values and we are reporting these determinations as made by the modified dry ashing method and by the Stevens' distilling method. Basal metabolic determinations were made on the same day that the samples of blood were drawn for analysis.

The following series of tests were run, using both methods on the same sample of blood and the end results were compared. It will be observed that the results obtained by both methods were in agreement.

Method employed. The dry ashing method was essentially that described by McCullagh (2, 3), while the distilling method employed in this work was the Stevens' (4) method as modified somewhat in this laboratory.

The apparatus as described by Stevens consists of a 500 cc. digestion flask, 10 cc. funnel, a connecting arm with ground glass joints and trap, and a condenser. For the receiver a 125 cc. Erlenmeyer flask is used and a Bunsen burner was substituted for the micro-burner since it was found that the volume of distillate could be kept constant more easily by this arrangement.

When the distillation is complete, the distillate is transferred to an S-tube.[1] The transfer is made carefully and with several washings of iodine-free water, the volume being kept at not more than 7 cc. Subsequent neutralization with 3% sulphuric acid, using 0.1% methyl orange as the indicator, was carried out in the tube with extreme caution (5). Bromine water was used in the oxidation in preference to bromine gas. The S-tubes are placed on the water-bath in the hood for 6 minutes to expel the bromine, after which the volume is concentrated to 1 cc. as shown by the graduation on the side-arm

Individual	Dry Ashing Method (γ per 100 cc.)	Distilling method (γ per 100 cc.)	B.M.R. %
(Normal male)			
Dr. G.	3.9	4.5	+ 2
Dr. H.	2.9	2.9	+ 2
Dr. P.	2.7	3.1	−17
Dr. M.	3.1	3.4	−18
(Normal female)			
Mrs. S.	3.5	3.3	− 7
Miss L.	2.3	2.5	+ 6
Miss S.	3.2	3.5	+ 1
Mrs. H.	2.9	3.4	− 2
Miss B.	2.4	2.2	−20
(Abnormals)			
Mrs. A. (Hyperthyroid)	16.0	18.0	+15
Mr. C.	6.4	6.3	+14
Mrs. S. (Acromegaly)	3.3	4.2	+ 2
Dog	3.4	3.8	

Comparative Table of Normal Averages

Method	Male	Female
Distilling	3.5	3.0
Dry ashing	3.2	2.8

of the tube. The solution is cooled in the refrigerator, after which 2 drops of 1% potassium iodide and 1 drop of 1% starch are added and the solution is titrated with .0005N sodium thiosulphate. The appearance of the blue color and subsequent end point of the titration is much more readily seen in this tube than in any other type of receptacle we have tried.

The S-tube referred to above is the one used by Strickler for titration in his dry ashing method. It is best described as a regular Pyrex test tube with a 5 cc. graduation and a side-arm graduated in 1 cc. This extends out at right angles at a point one inch from the bottom of the tube. A 0.2 cc. pipette graduated in .001 was used in the titration, the point of which was drawn to a small tip to prevent backdraw. The amount of potassium iodide added is of extreme importance since we found, in agreement with Allot, et al. (6) and with Perkin (7), that the addition of too much potassium iodide leads to erroneously high results.

[1] This tube was made for us according to Strickler's specifications by Mr. F. E. Donath at the U. S. Bureau of Mines at Pittsburgh.

DISCUSSION

The difference in the averages by the two methods in the male was 0.3 γ and in the female 0.2 γ. The range in the normals by the dry ashing method was found to be 2.4 to 3.9 γ, and by the distilling method 2.2 to 4.5 γ. Thus, the difference by the two procedures is so slight that it need not be regarded as a determining factor in the choice of a method.

The important consideration, since accuracy is equally well established by both methods, is that the Stevens' method is easier and more practical for routine laboratory work. It involves only two major steps and one transfer in the whole process. Only four reagents[2] are used in the distilling method up to the point of neutralization, whereas eight reagents are required in the dry ashing method and eight different transfers are performed. Consequently, it requires only two hours to determine blood iodine by the Stevens' method and fully one and one-half days by the dry ashing method, in addition to requiring the use of a muffle furnace.

A comparison of the basal metabolic determinations with the blood iodine values shows only the point that is generally recognized; namely, that the blood iodine is of value in hyperthyroid cases, but gives no indication of a hypofunctioning gland. Whether the basal rate is an indicator of the iodine content of the blood is not within the province of the present discussion.

CONCLUSIONS

A series of comparative determinations of blood iodine on the same sample of blood was done. No appreciable difference was observed in the values obtained by the distilling method as compared to those obtained by the dry ashing method.

The greater ease in performance, the saving of time, and the comparative decreased cost recommend the distilling method for the determination of blood iodine as the preferable method for routine laboratory work.

Slight modifications in procedure are suggested in Stevens' method in order to facilitate determinations.

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[2] In the dry ashing method some of the iodine-free reagents must be prepared, whereas in doing the distilling method we found DuPont's chromic acid flakes to be iodine-free, Baker's sulphuric and phosphorous acid and G. Frederick Smith's ceric sulphate to be satisfactory.

CASE REPORTS

CHRONIC IDIOPATHIC PARATHYROID TETANY

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THE PRESENT CASE of chronic idiopathic hypoparathyroidism is of interest for the following reasons. The condition is encountered relatively infrequently. Parathyroid hormone therapy was effective over an unusually long period of time (6 years). Vitamin D therapy was ineffective at all times. Increasing the phosphorus intake exerted a distinctly deleterious effect upon the subjective and objective manifestations and upon the effectiveness of parathyroid hormone therapy. No parathyroid tissue could be found at postmortem examination. Certain peculiar hemorrhagic features were prominent in the terminal stages.

CASE REPORT

The patient, *J. Q.*, a white girl of Irish descent, first entered the medical service of the hospital April 8, 1930, at the age of 6 years, complaining of convulsive seizures.

The family history was of no significance. She was born at full term and delivery was spontaneous and normal. The only points of significance in the past medical history were as follows. Measles at 2 years of age and tonsillectomy at 3 years. At 18 months the child, as a result of a fall, sustained a green-stick fracture of the right fibula, which healed completely after 1 month in bed. Shortly thereafter, a similar fracture was sustained in the left fibula, which also healed completely after about 1 month.

At the age of 3½ years (July, 1927), cramp-like pains were noted in the legs and arms, accompanied by stiffness of the extremities and difficulty in speaking. Since that time there has been a more or less constant sensation of stiffness in the arms and legs. In August, 1927, there was a severe convulsive attack, characterized by persistent spasm of arms, legs and face. These attacks lasted for several days and at times weeks, and recurred at intervals of 4 to 6 weeks. During the 6 months prior to admission there had been alternating diarrhea and constipation; on admission the stools were fairly well formed and otherwise normal.

Physical examination (4/8/30) showed a well-nourished child, very intelligent, of apparently normal skeletal and muscular development, with typical carpopedal and laryngeal spasm. The face presented the characteristic 'tetany facies,' with moderate pallor and puffiness of the skin. Strabismus occurred occasionally, the child complaining of transitory double vision. Chvostek and Trousseau signs were

elicited readily. There were severe nutritional disturbances in the dental enamel, nails and hair. The cathodal opening contraction was 1.0 milliampere.

The urine findings were normal; blood Wassermann reaction negative; red blood cells 3,250,000, Hb 75% (Dare), white blood cells 7300, with a normal differential count. The serum calcium was 4.8 mg. per 100 cc., and the inorganic phosphorus 7.4 mg., blood sugar 88 mg. and plasma CO_2 combining power 56 volumes per cent.

Roentgen-ray examination showed no evidence or rickets or other significant skeletal abnormality. The thymus appeared to be enlarged.

Prompt subjective and objective relief followed the intravenous and intramuscular injection of calcium gluconate. Calcium gluconate was given orally, 30 grains 4 times daily, 3 hours after meals, and a diet was given which was relatively low in protein and phosphorus. Parathyroid hormone, 1.5 cc. (150 U), was injected intramuscularly daily for one week. Smaller doses were found to be inacapable of controlling the condition. Investigation at this time revealed the fact that the child had received large doses of cod-liver oil and viosterol, with a high milk diet, constantly during the preceding 3 years without apparent benefit. Under the above regime clinical improvement continued without recurrence of symptoms. During the subsequent 3 years parathyroid hormone (1 cc., 100 U) was given at intervals of 1 to 2 days and the dosage of calcium gluconate was increased to 60 grains 3 times daily. This dosage of parathyroid hormone was found to be necessary for the prevention of tetanic manifestations. When the latter occurred, they subsided within 1 to 2 hours after injection of the hormone. The condition of the teeth, hair and nails improved markedly, these structures becoming essentially normal. The serum calcium and phosphorus concentrations during this period were as follows.

	Calcium	Phosphorus		Calcium	Phosphorus
	mg. per cent			mg. per cent	
4/ 9/30	4.8	7.4	8/22/31	10.24	5.4
4/13/30	7.95	5.6	10/14/31	9.24	6.1
4/28/30	11.31	6.3	12/15/31	8.9	6.0
5/11/30	10.74	6.2	2/20/32	9.3	6.2
5/18/30	9.78	6.5	4/15/32	10.25	6.3
10/14/30	8.74	6.2	8/16/32	9.42	6.2
12/16/30	8.8	6.0	10/12/32	9.64	6.1
1/28/31	9.03	4.5	11/ 8/32	9.78	6.3
4/22/31	9.31	4.7	1/ 4/33	8.62	6.6
6/18/31	9.78	5.3			

In May, 1933, at the age of 9 years, there was a recurrence of severe tetanic spasms which could not be controlled adequately by parathyroid hormone and oral calcium therapy which had been effective during the preceding 3 years. Careful questioning showed that for a period of about one month the child had been taking about 2 quarts of milk daily in addition to the diet previously prescribed. At this time (5/22/33) the serum calcium was 6.8 mg. per 100 cc. and the phosphorus 8.6 mg. The symptoms subsided promptly when the extra milk was omitted, the calcium and parathyroid dosage remaining the same. In 5 days (5/27/33) the serum calcium rose to 8.88 mg. and the phosphorus fell to 7.4 mg. One month later (6/23/33) the serum calcium was 9.64 mg. and the phosphorus 6.5 mg.

The condition continued to be well controlled under the former regime until May, 1934, when an attack of scarlet fever was associated with a recurrence of tetanic spasms which were relieved only by daily intravenous and intramuscular injections of calcium gluconate (serum calcium 7.12 mg. and serum phosphorus 8.1 mg.). These tetanic spasms were accompanied by bleeding from the gums and lips and by the appearance of subcutaneous extravasations of blood ($\frac{1}{2}$ to 2 inches in

diameter) on the legs, arms, face and chest. These hemorrhagic phenomena disappeared during convalescence, which was otherwise uneventful. After recovery from the attack of scarlet fever the condition was again readily controlled by the previously effective dosage of parathyroid hormone and oral calcium gluconate. In November, 1934, the serum calcium was 8.8 mg. per 100 cc. and the phosphorus 6.01 mg.

The patient was next seen in February, 1935, in a severe tetanic seizure. The serum calcium was 6.4 mg. and the phosphorus 7.7 mg. (2/24/35). During the preceding 2 months she had taken about 2 quarts of milk and 60 minims of viosterol daily (on advice of her physician) in addition to the usual amount of calcium gluconate (12 gm. daily) and parathyroid hormone (1 cc. daily). Carpopedal and laryngeal spasm of varying severity had existed almost constantly for the preceding 6 weeks. Cessation of extra milk and viosterol therapy resulted in prompt improvement. In about one week (3/2/35) the serum calcium was 8.6 mg. and the phosphorus 6.2 mg. per 100 cc., the dosage of parathyroid hormone and calcium being the same as formerly.

The child remained well until September, 1935, when subcutaneous hemorrhages began to appear spontaneously in the extremities in the absence of manifestations of tetany. The results of physical examination were otherwise negative. Other studies were as follows (9/9/35). Serum calcium 9.02 mg., phosphorus 5.8 mg., phosphatase activity 12.4 units (Bodansky). B.M.R.+12. Eye-grounds were normal; slit lamp examination showed no lenticular opacities. Roentgen-ray examination showed no evidence of skeletal abnormality and little if any evidence of demineralization. Red blood cells 4,260,000; Hb 81% (Dare); white blood cells 8700, with essentially normal differential; clotting and skin bleeding times normal; normal clot retraction; blood platelets 244,000; total serum protein 8.21 grams, plasma fibrinogen 0.299 grams per 100 cc.

The hemorrhagic manifestations continued to appear from time to time with occasional bleeding from the gums. In December, 1935, dystrophic changes began to appear in the hair, teeth and nails, increasing progressively during the next 3 months, although other manifestations of tetany were apparently controlled by calcium and parathyroid hormone therapy until March, 1936. The child was admitted to the hospital 3/10/36 after 24 hours of almost continuous generalized tetanic spasms. Severe trophic changes were present in the hair (dry, coarse, patchy alopecia), teeth (transverse ridging, enamel defects) and nails (soft, brittle, ridged and cracked). The liver edge was palpable 2 cm. below the right costal margin in the mid-clavicular line and the spleen was just palpable on deep inspiration. Trousseau and Chvostek signs were elicited readily. There were several old bruises in arms and legs. The temperature was 100°, pulse 110, respirations 18; laryngeal stridor was present; red blood cells 4,100,000; Hb 77% (Dare): white blood cells 10,000, normal differential; urine, albumin 1+, otherwise normal. The serum calcium was 6.21 mg. and the phosphorus 7.9 mg. per 100 cc.

Calcium gluconate, injected intravenously and intramuscularly, resulted in prompt relief from tetanic spasms which, however, recurred after 4 to 6 hours. For the first time since the patient came under observation (6 years) parathyroid hormone failed to control the tetany (except during periods of increased phosphorus intake). Injections of calcium gluconate at 6-hour intervals controlled the condition fairly well for about a week. Suddenly (3/18/36) the patient complained of severe abdominal cramp-like pain, accompanied by carpopedal and laryngeal spasm, uncontrollable bleeding from the gums and repeated hematemesis. The serum calcium (3/18/36) was 5.94 mg. and the phosphorus 8.1 mg. per 100 cc. The pain and tetanic spasms

were relieved by intravenous injections of calcium gluconate, but the bleeding and vomiting continued. On 3/24/36 the temperature rose to 103°.

The following hematologic data were reported by Dr. L. M. Tocantins (3/24/36): red blood cells 3,400,000; Hb 64% (Sahli); white blood cells 24,100, with essentially normal differential count except for an excessive number of stab forms of granulocytes; platelets 580,000; bleeding time 9½ minutes; venous clotting time 70 minutes; good clot retraction. Examination of a hanging drop preparation of the plasma (no added anti-coagulant) showed an abundance of platelets, which remained unagglutinated during a period of 5 days, at the end of which time only a few strands of fibrin could be seen. After several hours the platelets showed no Brownian movement and were remarkably evenly distributed throughout the preparation, although the red blood cells were in tight rouleaux formation. The addition of calcium to the plasma had no discernible effect upon these phenomena.

The patient received two transfusions of 260 cc. and 120 cc. of blood on 3/24/36, which had no influence upon the hemorrhagic manifestations (gums, hematemesis). Following the second transfusion the red blood cells were 1,900,000, Hb 42% (Sahli), white blood cells 47,100, platelets 320,000. The temperature was 105° and the child became stuporous and eventually comatose, with carpopedal and laryngeal spasm, the latter being relieved by parenteral calcium therapy. Parathyroid hormone was administered continuously during this period, apparently ineffectually. Shortly before death (3/25/36) the serum calcium was 6.18 mg. and the phosphorus 8.0 mg. per 100 cc. The lungs showed signs of edema and congestion, death occurring apparently as a result of circulatory failure. There were no localizing signs of intracranial hemorrhage.

AUTOPSY FINDINGS

Autopsy was performed 12 hours after death, examination being limited to the neck, thorax and abdomen. The body was that of a well-developed, fairly well-nourished female child. There was no gross evidence of rickets or other skeletal abnormality. There was only slight post-mortem rigidity and lividity. Surrounding several needle punctures in the skin of both thighs and right antecubital space were round purpuric spots, about 4 cm. in diameter. There were no petechiae in the lips, mouth, tongue or conjunctivae. Elsewhere, the skin presented a pale, marbled appearance suggestive of severe anemia. No edema or jaundice. The teeth showed transverse ridging of the enamel and the nails were markedly atrophied, with peronychiae. There were patchy areas of hair loss in the scalp and eyebrows. The breasts were undeveloped and there was no axillary or pubic hair. There was little subcutaneous fat and the musculature was pale.

The pleural, pericardial and peritoneal surfaces were smooth, glistening and free of adhesions, and the serous cavities showed no excess of fluid. Both parietal and visceral surfaces of the pleura were thickly studded with hemorrhagic areas ranging in size from minute petechiae to large purplish or brownish-red spots 3 to 4 cm. in diameter. There were a few petechiae in the parietal peritoneum and extensive areas of hemorrhage on both sides of the vertebral column in the abdomen and at the root of the mesentery. The hemorrhagic lesions were smaller and less numerous in the visceral and parietal pericardium.

The heart weighed 170 gm. The musculature was of good tone and showed no gross abnormality. The aorta appeared slightly smaller than normal. Microscopically the heart and great vessels showed no significant change.

Both lungs were congested and edematous throughout and there were patchy areas of hemorrhage in the lower lobe of the right lung. Microscopic examination

revealed extensive edema and scattered areas of hemorrhage and bronchopneumonia.

The right kidney weighed 100 gm.; on section, the cortex was pale and the pyramids dark. The left kidney (150 gm.) showed considerable hemorrhage into the perirenal space, beneath the capsule and around the calyces and pelvis. Both organs were otherwise normal grossly. The glomeruli and tubules were essentially normal except for slight tubular epithelial degeneration and the presence of a few hyaline casts.

The right adrenal gland appeared normal. The left adrenal was embedded in hemorrhagic fibro-fatty tissue. The extravasated blood was densely infiltrated with leukocytes. The adrenal glands showed no histologic abnormality.

The urinary bladder was normal. The uterus, fallopian tubes, ovaries and vagina were perhaps slightly smaller than normal for a child of this age, but were otherwise normal.

The esophagus was normal. The stomach was dilated and contained a large amount of coffee-ground material. No petechiae were observed in the mucous membrane of the stomach or duodenum. Beneath the serous surface of the jejunum there were scattered brown hemorrhagic spots, less than 1 cm. in diameter. A single hemorrhagic area was present in the peritoneum of the sigmoid. Scattered mucosal petechiae were present in the jejunum, ileum, cecum and sigmoid. The intestines contained coffee-ground material. Microscopic examination showed no abnormality other than the hemorrhagic lesions mentioned above.

The liver weighed 1080 gm. and measured 25—18—6 cm. The capsule was normal; the edges were slightly rounded. The cut surface was red, firm and the lobular markings somewhat indistinct. The gall bladder and extrahepatic bile ducts were normal. Microscopic examination showed an excess of connective tissue in the portal areas, extending into the interlobular areas and partially surrounding the lobules. Much of this fibrous tissue was hyalinized, with occasional areas of infiltration of small round cells and small proliferating bile ducts. The hepatic parenchyma was well preserved except for degeneration and necrosis of a few cells near the interlobular connective tissue and the portal areas. There was little evidence of invasion of the lobules by connective tissue and, aside from the perilobular fibrosis, the normal architecture of the hepatic parenchyma was well preserved.

The spleen weighed 110 gm. The cut surface was firm and dry. The splenic pulp was abundant and cellular; the primary follicles were small, with relatively large secondary centers, containing large mononuclear cells and a few polymorphonuclear leukocytes.

The pancreas and skeletal muscle were pale but otherwise normal. Lymph nodes from various situations were normal. The thymus weighed 10 gm. and showed a few pin-point petechiae in the capsule; it was normal microscopically. Permission to examine the brain, spinal cord and bones was expressly denied. The marrow of the sternum and ribs was essentially normal.

The thyroid gland, weighing 15 gm., was dissected from the surrounding structures with its capsule intact. Careful gross examination of the thyroid gland, neck and mediastinum, pericardium and thymus showed no evidence of structures that might possibly be parathyroid glands. The thyroid gland was placed immediately in 10% formaldehyde solution and was allowed to fix for 24 hours. Small pieces of tissue (1 to 2 mm. thick) were cut from the posterior portion of each lateral lobe. In this manner the entire area of the thyroid gland in relation to the ordinary location of the parathyroid glands was cut into thin pieces and examined carefully under the hand lens. The remainder of the gland was then examined in a similar manner. No

evidence could be detected grossly of the presence of structures resembling parathyroid glands. These small pieces of tissue were then blocked in paraffin and numerous histologic sections were cut from each block at different levels. With the procedure employed, parathyroid glands, if present, would have to be of minute size to have escaped detection. However, in none of the many sections examined was there any structure resembling parathyroid gland or any group of cells possessing the characteristics of the cellular components of these glands. The thyroid gland was normal. The acinar epithelium was of the low form, colloid was normal in amount and appearance, and the acini showed very slight variation in size.

Pathological Diagnosis: a), Absence of parathyroid glands. *b*), Chronic hepatitis. *c*), Bronchopneumonia. *d*), Extensive hemorrhages into the subcutaneous tissue, viscera, serous and mucous membranes and retroperitoneal space. *e*), Anemia. *f*), Follicular hyperplasia of spleen. g), Dystrophic changes in teeth, hair and nails.) *h*), Mild degree of renal tubular degeneration (nephrosis).

DISCUSSION

The occurrence of tetany with hypocalcemia and hyperphosphatemia, the absence of roentgen-ray evidence of skeletal abnormality and the failure to find any trace of parathyroid tissue at post-mortem examination leave little doubt that this case is one of true idiopathic hypoparathyroidism. The cause of the condition cannot be stated. Unfortunately, permission to examine the pituitary gland was denied. However, the normal condition of the adrenal and thyroid glands, and the essentially normal condition of the ovaries, tubes and uterus are strong, although not conclusive, evidence against the existence of an underlying state of pituitary hypofunction. Although no parathyroid tissue could be found at autopsy, it seems inconceivable that these glands were congenitally absent in view of the fact that the child developed normally and presented no manifestations of parathyroid deficiency during the first three years of life. The possibility must be considered of progressive atrophy or degeneration and eventual disappearance of originally normal or hypoplastic glands. The question arises as to whether the prolonged administration of large amounts of parathyroid hormone could have contributed to such atrophy. Jaffe and Bodansky (1) observed a decrease of about 50% in the size of the parathyroid glands of previously normal dogs dying of acute experimental hyperparathyroidism. This was regarded as evidence of functional involutional atrophy due to substitution therapy. Pappenheimer and Johnson (2) were unable to obtain similar results in rats. Their findings must be interpreted with caution because of the variable response and apparent relative resistance of this species to parathyroid hormone. It is questionable, however, whether such an effect can occur in the case of already hypofunctioning glands when only enough hormone is supplied to maintain normal function. No information is available bearing directly on this question, which must remain unanswered for the present.

There are several points of interest in connection with the response to treatment in this case. It is frequently stated that the influence of parathyroid hormone upon the manifestations of tetany is exerted rather slowly, in accordance with the observation by Collip (3) and others that the consequent

rise in serum calcium (in normal dogs) occurs gradually, reaching a maximum in 15 to 18 hours. It is believed by some (4, 5) that parathyroidectomy (dog) increases sensitiveness to the hormone, but this is denied by Collip and Clark (6). However, we (7) have observed that the rise in serum calcium in normal human subjects is more rapid, reaching a maximum in about 6 hours after intramuscular injection of the hormone. We (8) have also found a similarly rapid response in normal dogs maintained upon a diet low in phosphorus. It has been our experience that, as noted by Lisser and his associates (9), the subjective manifestations of tetany may be relieved promptly (within 30 minutes) following injection of the hormone, followed (within a few hours) by improvement in objective manifestations even though the serum calcium concentration may not be significantly elevated until later. In the present case, in which parathyroid hypofunction was obviously of extreme degree, distinct improvement occurred invariably within 1 to 2 hours after intramuscular injection of adequate amounts of the hormone. The recent observations of Cantarow, Haury and Whitbeck (10) regarding the influence of the hormone upon the in vivo diffusion of calcium and phosphorus may have some bearing on this problem. Whatever the reason may be, it seems certain that the rather prompt response to treatment cannot be explained solely on the basis of increase in the serum calcium concentration. The decrease in serum phosphorus may be of importance in this connection.

One of the most significant features of this case, in our opinion, was the obviously deleterious effect of an increased phosphorus intake. As stated by Shelling (11), despite the frequent demonstration of the importance of phosphorus in tetany, little attention is paid to this element in the dietary treatment of parathyroprivic tetany in human beings. In a discussion of this subject, Shelling and Goodman (11) point out that this lack of appreciation of the relation of phosphorus to parathyroid function may account for the occasional failure of calcium and parathyroid hormone therapy in parathyroprivic tetany and for the occasionally reported observation that the hormone loses its effectiveness after repeated injection over long periods of time. Several examples of such apparent refractoriness were analyzed by these authors and the probable importance of this factor demonstrated. Certain other pertinent observations are of interest. Albright, Bauer, Claflin and Cockrill (12) found that the administration of phosphates to patients with hyperparathyroidism resulted in a decrease in serum calcium concentration and an increase in serum phosphorus. Similar changes have been observed frequently in normal animals following the intravenous injection of phosphates. Robbins and Kydd (13) reported a decrease in urinary calcium excretion following the addition of phosphate to the diet. Allardyce (14) found that the response to parathyroid hormone was slight and irregular on a diet consisting chiefly of rolled oats (high phosphorus content) and was not improved by adding cod-liver oil or calcium. These observations support the view that an increased phosphorus intake tends to exaggerate the metabolic defect characteristic of the hypoparathyroid state and may be responsible for the occasional supposed 'refractoriness' to the hormone exhibited under such circumstances. The fact is too frequently overlooked that milk, which is usually administered

in large amounts in these cases, provides an excess of phosphorus as well as of calcium, and may therefore, as emphasized by Shelling and Goodman (11), actually exert a deleterious effect in parathyroprivic tetany. This appeared to be true in the present case. The tetanic state was uncontrolled for a period of about 3 years during which the child received large amounts of milk and vitamin D (cod-liver oil and viosterol). Parathyroid hormone was consistently effective in maintaining an essentially normal serum calcium concentration and in controlling the tetany during the next 5½ years, in conjunction with a high calcium and relatively low phosphorus intake. This regime became ineffective on two occasions when the only change consisted in an increased intake of phosphorus (and calcium) and vitamin D, and again became effective when the latter were omitted.

There has been considerable controversy regarding the value of vitamin D therapy in parathyroprivic tetany. It is well known that this agent is usually capable of causing an increase in serum calcium from tetanic to normal levels and has been successfully employed for this purpose; however, the possible dangers attending its use have been stressed by Shelling and his associates (15, 16), who direct attention to the simultaneously increased phosphorus retention and increased serum phosphorus concentration. The theoretical aspects of this problem, and certain important differences between the physiologic effects of vitamin D and parathyroid hormone have been reviewed elsewhere (17, 18). Suffice it to state that we subscribe to the view expressed by Shelling and Goodman (11) that vitamin D should be used cautiously, if at all, in the treatment of parathyroprivic tetany. Inability to control this type of tetany by this agent has been reported by these observers (11), Leopold and Jonas (19) and Liu (20), and was a conspicuous feature in the present case. Unfortunately, dihydrotachysterol was not available at the time this patient was under observation. Recent metabolic studies (21) as well as several clinical reports indicate the usefulness of this substance as a substitute for parathyroid hormone.

We feel that the ineffectiveness of the hormone in the last few days of life in the present case may have been due to the presence of fever, which, as pointed out by Shelling and Goodman (11), may result in the liberation of considerable amounts of phosphorus from tissue breakdown. The deleterious influence of fever upon parathyroprivic tetany has been noted frequently. Death was due apparently directly to the peculiar hemorrhagic state which manifested itself in mild form during the last year of life and acquired a fulminating character during the last week. The exact nature of this state is questionable. In certain respects the condition of the blood resembled that in hemophilia and anaphylactic shock. There was no basis for, nor evidence of, scurvy and the hematologic findings were not those of that condition or of purpura hemorrhagica. Reviewing these findings in the light of present knowledge, they appear to resemble a state of prothrombin deficiency more closely that any other disorder. There was no apparent basis for the development of such deficiency with the possible exception of the rather mild chronic hepatitis, a possibility that seems rather remote. We have never encountered any such hematologic phenomena in a rather extensive experience with para-

thyroid administration and are not familiar with any similar reports in the literature. We believe it unlikely that these phenomena were a direct consequence of either tetany or the administration of the hormone, although no definite statement can be made in this regard at the present time.[1]

The cause of the hepatic lesion is likewise doubtful. Larson and Elkourie (22) reported hepatic cell and renal tubular necrosis in dogs dying of parathyroprivic tetany. The nature of this relationship is difficult to understand, but it is possible that the state of chronic hypoparathyroidism may have been related in some way to the development of chronic hepatitis in the present case.

SUMMARY

A case is reported of severe, chronic idiopathic hypoparathyroidism which did not respond to vitamin D therapy and was effectively controlled by large doses of parathyroid hormone and a high calcium, relatively low phosphorus intake over a period of 5½ years. This regime became ineffective on two occasions, when the only change consisted in an increased intake of phosphorus (and calcium) and vitamin D, and again became effective when the latter were omitted. The significance of this phenomenon is discussed. Death was due apparently to the development of a peculiar hemorrhagic state, with extensive hemorrhages throughout the body. The exact nature and pathogenesis of this state could not be determined; it may have been dependent upon a condition of prothrombin deficiency.

No parathyroid tissue could be found at autopsy. Aside from the hemorrhagic manifestations, the only other significant findings were a mild chronic hepatitis, mild nephrosis, bronchopneumonia and trophic changes in the hair, nails and teeth.

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[1] Greenberg, Boelter and Knopf (23) recently reported a peculiar hemorrhagic condition occurring in rats maintained upon diets very low in calcium. The possible relation of this condition to that which was present in the case under consideration cannot be stated at the present time.

A CASE OF PITUITARY DWARFISM TREATED WITH ANTUITRIN-G

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THIS CASE is reported to demonstrate the therapeutic effect of antuitrin-G on growth in a pituitary dwarf, on whom the causal factor of dwarfism was active. Pituitary dwarfism may be due to an idiopathic insufficiency of the eosinophilic cells of the anterior lobe of the hypophysis, or result from pressure upon these cells, with subsequent atrophy. The pressure may be produced by an overgrowth of other types of cells in the pituitary gland, as a chromophobic or basophilic adenoma. The pressure upon the gland may also result from increase in size of surrounding structures, as tumors or inflammatory lesions in the hypothalamic area. Cases illustrating these various causal factors are described by Cushing (1), Bailey (2), Weber (3), Dott and Bailey (4), Mott and Barrett (5), Beckmann and Kubie (6), Critchley and Ironsides (7), and Zeitlin (8), as well as others.

Chromophobe adenomata in childhood and adolescence are rare. Basophilic adenomata are more common. Craniopharyngioma, a type of extra-pituitary tumor, may produce dwarfism by pressure upon the pituitary and hypothalamic structures. This type of tumor frequently contains cholesterin crystals. Weber (3), Critchley and Ironsides (7), and Zeitlin (8), have reported such tumors. Holloway (9) has compared the ophthalmological findings in these tumors with those observed in true pituitary tumors, and finds that in both types of tumor, headache and failing vision are the usual early symptoms. In pituitary adenomata, there was bitemporal hemianopsia in 75% of the cases, homonymous hemianopsia in 11%, and concentric contraction of the visual field in 2%. In the adamantinomata, bitemporal hemianopsia was encountered in 50%, homonymous hemianopsia in 27%, and concentric contraction of visual fields in 18% of the cases. Patients with adamantinomata revealed papilledema in 53% of the cases, primary optic atrophy in 46%, while 84% of pituitary adenomata exhibited optic atrophy, 9% papilledema, and 6% had normal discs.

The true pituitary dwarf exhibits a general bodily underdevelopment, with aplasia of the genitalia, and non-appearance of hair in those loci normal to developing youth. The children may be obese, and most of them retain the the high-pitched voice of childhood.

Evans (10) believes it possible that all cases of human dwarfism are directly caused by inadequate pituitary function. He bases this opinion on the fact that the restoration of growth in cretinic dwarfs may be effected by either administration of thyroid extract or direct treatment with pituitary extracts, indicating that the pituitary function is depressed as a result of

thyroid subnormality. We report the effects of antuitrin-G, the growth promoting substance, in a well studied case of pituitary dwarfism from pressure, who had previously received adequate thyroid therapy without results.

Case Report

The patient, an 18-year-old, undersized girl was first admitted on January 16, 1937. (fig. 1, 2, 3). She weighed 30 kg. (65 lb.), and was 129 cm. (51⅝ in.) in height. Failure of growth had not seemed unusual to the parents, who were both of short stature. The height of the father was 158 cm. (63 in.), mother 145 cm. (58 in.), a brother 168 cm. (67 in.), and one sister 150 cm. (60 in.). There were three other siblings of normal height. The fact that the patient had menstruated only once, in August, 1936, for 7 days without pain, had not impressed the parents. The patient suffered from frontal and occipital headaches, and pain at the 'top of her head,' which had begun 2 years previously. She had occasional attacks of dizziness and vomiting, unaccompanied by nausea, after meals. Some months before admission an attack of unconsciousness occurred, in which the patient fell to the floor without a cry and remained in a tonic convulsion for about 15 minutes, during which time she bit her lip. The convulsion was followed by a deep sleep. There was no involuntary passage of urine nor feces. Shortly before admission to the hospital she had had two more convulsive seizures, following which she lost 7 lb. in weight.

Her past history revealed measles and pertussis in childhood. She had been a good student, but had been removed from high school because of her illness. She used her eyes very little as any visual task caused pain in the eyeballs. She preferred female to male companions and had no sexual urge. Her mental interests were those of an 11-year-old child.

On physical inspection, the patient presented the usual picture of a normal individual, but appeared much younger than her actual age. The eyeballs were prominent. She was symmetrically proportioned and the panniculus adiposus was normal in amount and distribution. The physical examination was normal including the distribution of genital hair. There was an excess of hair on the forearms. Axillary hair was not present. The vision was essentially normal. There was no nystagmus nor strabismus. The fundal veins were somewhat dilated, but there was no papilledema.

The patient's body measurements on admission are given below and may be compared, where data are available, with the measurements accepted as normal for an 18-year-old girl of normal height (161 cm.), and normal weight (54.6 kg.)

	Patient	Normal (Available Comparative Measurements[1])
Head—O.F.	55 cm.	
S.O.B.	53 cm.	
Height	129 cm.	161 cm.
Arm length	27 cm.	
Forearm length	22 cm.	
Hand	15½ cm.	
Wrist	13 cm.	
Chest—relaxed	64½ cm.	
expanded	67 cm.	72.8 cm.
Waist	57 cm.	
Thigh length	34 cm.	
Leg length	35 cm.	
Total length of lower extremity	69 cm.	75.2 cm.
Foot—plantar surface	21 cm.	

[1] Comparative measurements through courtesy of H. V. Meredith, Research Associate in Physical Growth, The State University of Iowa, Iowa City, Iowa.

Roentgenograms showed that the epiphyses of the femora, tibiae, fibulae, acromions, coracoids, and humeri were not closed (fig. 4), but were normal for a person of her age. A plate of the skull in the right lateral position showed thinning of the calvarium, erosion of the anterior clinoid process of the sella turcica, an en-

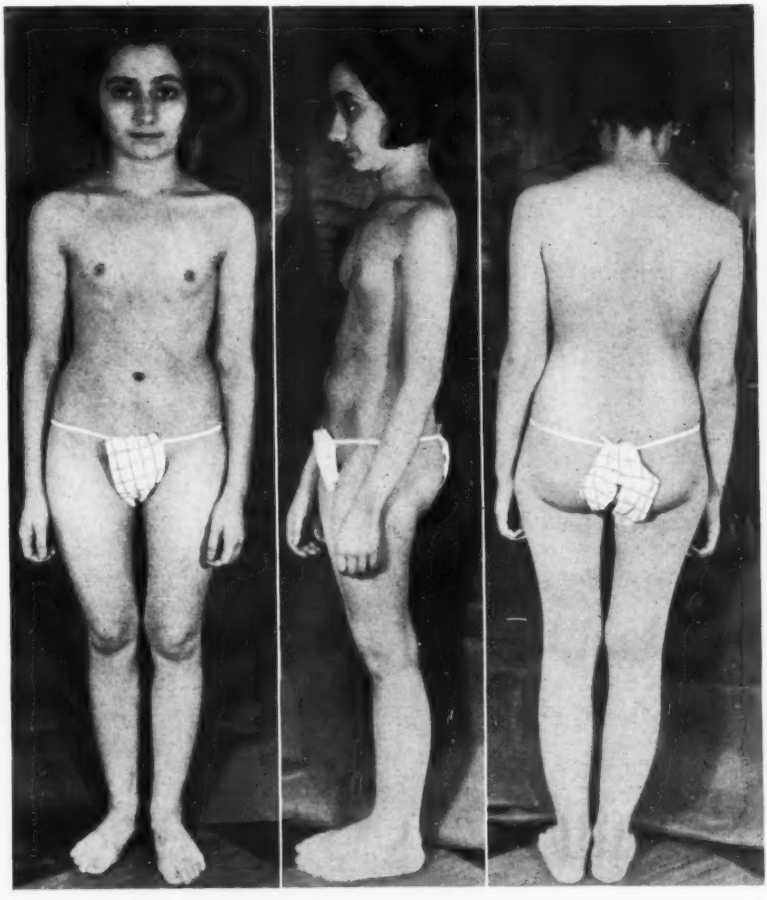

Fig. 1, 2, 3. Illustrates frontal, lateral and posterior appearance of patient. The eyes are prominent.

largement of the sella turcica, and calcification within the area occupied by the pituitary gland. Kornblum (11), found the normal measurements of the sella turcica in the average adult skull to be 8 mm. in depth and 10 mm. in anteroposterior diameter, with 10 mm. and 12 mm. being considered the upper limits of normal. In our case the sella measured 14 mm. in both depth and anteroposterior diameter (fig. 5).

The results of a urine analysis were within normal limits. The red blood cell count was 3,750,000 with 74 per cent hemoglobin. There were 8600 leucocytes, of

which 64 per cent were polymorphonuclear cells and 36 per cent lymphocytes. The blood Kahn and Wassermann tests were negative. The blood chemistry showed: cholesterol, 190, phosphorous, 6; and calcium, 10 mg. respectively in each 100 cc. of blood.

A diagnosis was made of dwarfism due to a pituitary tumor with hypofunction of

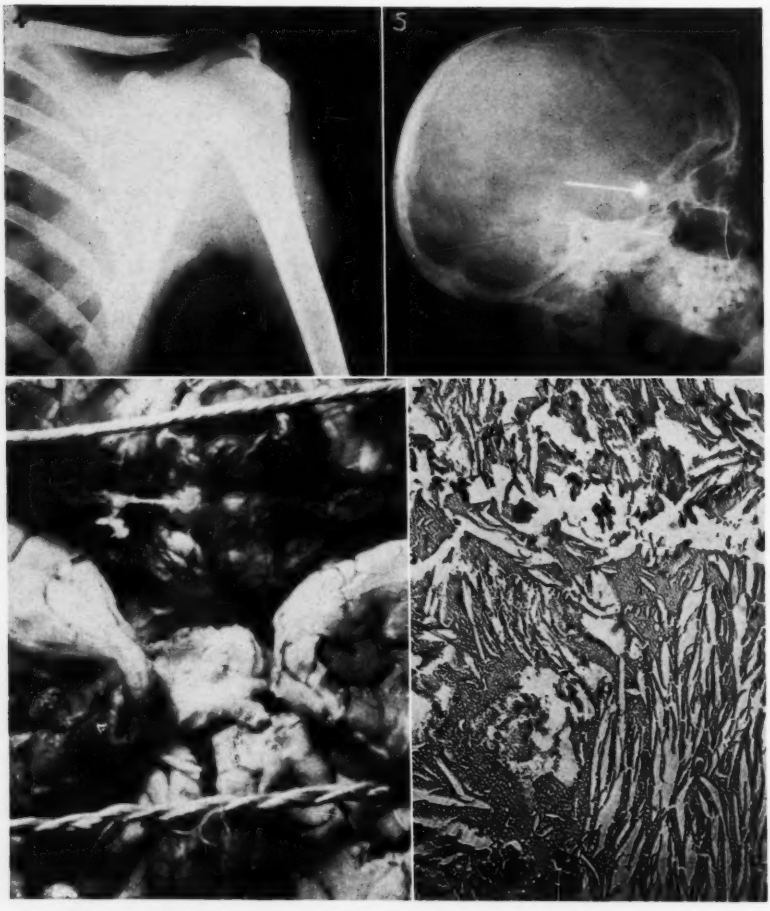

FIG. 4. SHOWS THE OPEN EPIPHYSES OF THE ACROMIAL PROCESS OF THE LEFT CLAVICLE. Fig. 5. DEMONSTRATES CHANGES IN THE SKULL AND IN THE SELLA TURCICA. Fig. 6. THE TUMOR LOCATION IS SHOWN. Fig. 7. SHOWS SPACES FORMERLY FILLED WITH CHOLESTERIN CRYSTALS. The dark areas express calcium deposition. (×45)

the growth and gonadotropic hormones. Clinical evidence expressive of change in any of the other accessory glands was absent.

The therapeutic possibilities were explained to the parents. They would not consent to surgical procedures nor radiation. The case presented an opportunity to determine the growth power of antuitrin-G (Parke, Davis & Co.). The patient, there-

fore, was given 1 cc. daily for 2 days and then 2 cc. daily for the next 12 days. The hospital residence ended January 31, 1937. The nausea, vomiting, and headaches were less severe at the time of discharge.

Extra Mural Course

After leaving the hospital, the patient was under constant observation. On the first visit, she weighed 29 kg. (63½ lb.), and was 129 cm. (51 5/8 in.) tall. She had not grown in height since her eleventh year. The headaches were less marked, and there had been no more attacks of dizziness. The appetite had increased markedly, but she complained of stiffness in the lower jaw. Vitamins B, C and E were added to the diet in large quantities, and the antuitrin-G was continued in daily 2 cc. doses. After two weeks she began having lower abdominal pains as if she were going to menstruate, but the catamenia was not established. She was again seen on March 19, 1937. For the previous two weeks she had been having a considerable amount of pain in the back of the neck. When the pain was severe she would vomit without nausea. She weighed 30 kg. (66¾ lb.), and was 131 cm. (52½ in.) tall. She complained of cramp-like, lower abdominal pains at times. She appeared stronger, was much more playful and used her eyes with less discomfort.

Between March 19, and April 9, 1937 she vomited twice. Her ocular fundi were normal at this time and her breasts showed some definite development. From then until May 5, 1937, she had one severe occipital headache. For the first time in over a year she had gone to the cinema and was able to observe the picture without orbital pain. The weight was 30 kg. (66 1/8 lb.), and she was 131 cm. (52½ in.) tall. Axillary hair had begun to appear. On May 9, 1937, the catamenia was established and lasted a few hours.

On May 26, 1937, she weighed 31 kg. (69½ lb.). There was a very slight increase in height, and a wisdom tooth had erupted. Her breasts were larger and axillary hair was growing. Headaches were fewer and she felt able to go out of doors occasionally.

During the next two weeks there was no distress. On June 16, 1937, she weighed 32 kg. (71 lb.) and was 132 cm. (52 7/8 in.) tall. There was less hair on the arms and the breasts had further increased in size.

During the next month she had only one headache. She had been sewing and reading a good deal without orbital pain. She now weighed 34 kg. (75 1/8 lb.), but had not increased in height.

On August 13, 1937, she stated that she had had a very slight menstrual flow and had had leukorrhea for several days. Her weight had increased to 35 kg. (77 7/8 lb.). The height was unchanged.

On September 14, 1937, she complained that she had been having headaches for the last 6 days, had vomited once and was dizzy at times. She was unable to sleep because of pain in the back of the neck. She now weighed 36 kg. (80 lb.) and her height was 132 cm. (52 7/8 in.).

During the next month she had headaches every day, had vomited at times and was dizzy frequently. She had severe pains over the lower abdomen nearly every day, and had troublesome leukorrhea. She had gained ¾ of a pound, but her height remained stationary.

On November 3, 1937, a severe headache developed, with pain in the back of the neck, retraction of the head and stiffness of the neck. She vomited a great deal and was very restless. The next day she was better. On November 5, 1937, she vomited a few times, but felt quite well. The nuchal stiffness had disappeared. On November 6, the headaches were again severe, the neck rigid, and the temperature was 101° F. She was admitted to the hospital.

During all these months the antuitrin-G had been continued, but the intervals of administration were increased first to 2 and then to 3 days. All routine laboratory examinations were negative during this period, and she had gained 8 kg. (17¼ lb.) in weight, and 3 cm. (1¼ in.) in height.

She re-entered the hospital on November 6, 1937, with the complaints of headache, dizziness, nausea and vomiting. She appeared well nourished. She was stuporous, but could be aroused to answer questions. The mental reactions were normal. She rested on the left side with her head markedly extended and her neck rigid. Knee and ankle jerks were hyperactive. There was bilateral ankle clonus, but none of the signs designated as Babinski, Gordon, Oppenheim, Kernig and Brudzinski were present. The temperature was 101° F, pulse 120 and respirations 24. A spinal tap was done and 20 cc. of clear fluid was removed under slightly increased pressure. The analysis of the fluid showed no abnormalities.

The next day the patient was mentally alert. The signs of meningeal irritation were no longer present. She sat up in bed, and did not complain of headache nor dizziness. She ate well, and spoke to her visitors in a normal manner. She was, however, incontinent of urine. Toward evening she began to complain of a pain in the back of the head and neck. She slept fairly well that night, but awoke the next morning with the same pain, nausea and vomiting. About 8:45 A.M. she was given an enema which was expelled in the usual manner. A short time later she had a convulsion in which she rolled her eyes, moaned, and perspired profusely while foaming at the mouth, and became unconscious. She became very cyanotic, the heart sounds faint, but not rapid. Death occurred at the end of 30 minutes.

A postmortem examination was made. It showed a body of a young white female weighing about 80 pounds and about 4 feet 5 inches in height. The scalp was incised and reflected, and the calvarium was found to measure less than one-eighth of an inch in thickness in the frontal and occipital regions. The convolutions on the superior surface of both cerebral hemispheres were flattened. The vessels on the surface were moderately congested and tortuous. The brain was removed. It was larger than normal, weighing 1300 gm. A depression was present at the base of the brain with tumor involvement in the region of the pituitary gland. (fig. 6). Serial section of the brain showed the presence of a gelatinous tumor mass involving the floor of the third ventricle and extending down to and including the pituitary gland. There was an area of softening extending from the left lateral wall of the left ventricle towards the thalamus on the left side, but not actually destroying it. There was a marked dilatation of the lateral ventricle. The sella turcica was widened and lengthened and deepened with erosion of the clinoid processes.

Microscopic sections were taken, and although seen by several pathologists, no agreement nor any definite diagnosis was reached. In general, the sections showed fibrosis, cholesterin crystals, foreign body giant cells and small clusters of apparently young, undifferentiated cells. The opinions given varied from chronic inflammation with deposition of cholesterin to neoplasm of undetermined type (fig. 7).

SUMMARY

This case presents a type of pituitary insufficiency due to external pressure on the hypophysis and hypothalamic structures by a slowly growing tumor mass. Surgery and irradiation were refused. Antuitrin-G was given to determine its efficacy. Inasmuch as there had been no change in height, nor weight, nor physical appearance for seven years, there was a definite

response to the growth properties of this medication, shown by an increase in height of 3 cm. (1¼ in.); and increase in weight of 8 kg. (17¼ lb.); appearance of axillary hair; the establishment of the catamenia; and enlargement of the breasts. The decrease for some time in the severity and frequency of headaches, vomiting, nausea, dizziness and orbital pain was undoubtedly due to variation in size of tumefaction and not directly related to the therapy.

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HAIR GROWTH FOLLOWING THE REMOVAL OF A HYPERNEPHROMA

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THAT HAIR growth is dependent upon hormone influences is evident from the hirsute anomalies that are seen in various endocrinopathies. The appearance of the masculine hair pattern in women suffering from arrhenoblastoma or tumors of the suprarenal cortex and, in lesser degree, after the climaceteric, may be cited. Tumors of the pineal or anterior pituitary gland are also accompanied in some cases by abnormalities of hair growth. The scantiness of the hair of the scalp, eyebrows and body frequently seen in hypothyroidism and abnormalities of the hair patterns in gonadal deficiency are also evidences in point.

It is desired to place on record the case of a man in whom the removal of a hypernephroma was paradoxically followed by partial correction of complete hairlessness.

The patient was first seen at the age of 68 in October, 1931, after an attack of influenza. He stated that a few years before he had lost all of his hair but without having experienced any symptoms of bodily disorder. Physical examination disclosed no directly relevant findings. There was soft enlargement of the liver; the thyroid was not detectable by palpation and Chvostek's sign was absent. The pulse rate was 76, the blood pressure 150 mm. Hg.

Fig. 1

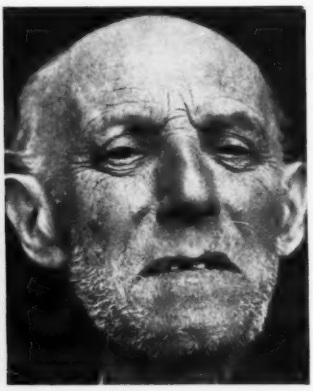

Fig. 2

In February, 1932 the patient experienced hematuria and urological examination disclosed a tumor of the left kidney. This was removed by Professor G. Illyes. Pathological examination was made by Professor K. Buday. The kidney itself was not enlarged and the tumor was about a third the size of the kidney. The renal vein was full of a red mass of flesh-like consistency. On section the tumor was seen to have the typical structure of a hypernephroma. The tumor cells were arranged in long narrow columns, mostly

parallel. Some of the columns had central lumina which were dilated and filled with either homogenous colloid material or red blood cells. The mass from the renal vein consisted entirely of recently developed tumor tissue.

Figure 1 shows the condition of the patient prior to operation. The absence of eyebrows and scalp hair is evident. Two weeks after the operation new growth of hair began to appear sparsely on the head and face and gradually increased. The cachexia which he had shown previous to operation disappeared and he became well-nourished and enjoyed general good health until 1936. He then began to experience pain in the back and to deteriorate mentally and bodily. At this time a skiagram disclosed a tumor mass invading the second and third lumbar vertebrae.

Figure 2 shows his appearance a year later. The growth of the eyebrows and of a considerable amount of scalp hair and of beard is evident. The photograph fails to bring out clearly the mustache which was also present. The amount of hair was approximately the same as the patient had had at the age of 55, twenty years before.

The case presents two paradoxes. Tumors of the adrenal cortex, when they influence the hair growth at all, usually give rise to the masculine pattern. Presumably, this effect is due to an accentuation of the androgenic principle acting either directly or through the primary gonads. Yet in the patient described, it was removal of the tumor that was followed by renewed hair growth and this of the masculine pattern. Such a happening in a male suggests the existence not of a masculinizing but of a feminizing adrenocortical influence. That such an influence must now be recognized is, however, indicated by the occurrence of a rare anomaly as in the case reported by Lisser[1] in which an adrenal tumor was accompanied by feminization of the subject. The second paradox lies in the fact that the development of the lumbar tumor, which is not considered as metastatic but as a local recidive issuing from the rests along the renal vein, did not reverse the effect which followed removal of the tumor initially. This latter fact needs the auxiliary assumption that the recidive tissue has lost the hormonal potentiality of the original tumor. However, one must take into consideration the fact that complete hairlessness is not characteristic for women nor for feminization.

There is also the possibility of interpretation without supposing an endocrine effect of the hypernephroma namely the pressure and therefore the local troubling effect of the primary tumor at the function of the left suprarenal gland. But this interpretation needs the auxiliary theory of the right gland being asplasic or its function influenced by some toxic effect.

SUMMARY

The case is described of an aged man who had been for several years completely hairless and in whom the removal of a hypernephroma was followed by the growth of scalp, and bodily hair. Five years later a recidive tumor of the vertebral column was recognized but the hair growth persisted. These events are compatible with a theory that either adreno-cortical tumors, although usually exerting a masculinizing influence can also produce a feminizing hormone, or that the pressure of the tumor on the left suprarenal gland inhibited its endocrine function.

[1] Lisser, A. *Endocrinology* 20: 567. 1936.

CLINICAL PATHOLOGICAL REPORT OF A PATIENT RECEIVING LARGE DOSAGE OF ESTROGENIC HORMONE

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IT HAS BEEN demonstrated that the hormones of the anterior lobe of the hypophysis and the ovaries exert a mutual effect upon each other (1). In the involutional period the activity of the anterior lobe of the pituitary has been found to be definitely increased (2). When estrogenic hormone is given to these women in the involutional period there is found to be a definite decrease in the excretion of gonadotropic hormone which seems to point to the inhibiting effect of the ovarian hormone upon the anterior lobe of the pituitary. Kirklia and Wilder (3) demonstrated that the somatotropic function of the pituitary was depressed by follicular hormone in certain acromegaloid individuals at menopause, which indicates that the action of follicular hormone on the pituitary is not limited to the depression of a single function. Although we have some insight into the action of these hormones, very few pathological reports have been published dealing with human material after large doses of hormone have been given.

The present clinical pathological report concerns a woman with the syndrome of involutional melancholia who was given large doses of estrogenic hormone and died 6 weeks later of other diseases. The report on the pituitary is of interest if for no other reason than the relatively negative findings after large doses of estrogenic hormone.

Clinical Case History. The patient was a 48-year-old married housewife. There was no mental disorder in her family. The patient had had one previous episode of agitation and depression occurring at the onset of her menses when she was 13 years of age. She was in a state hospital for 6 months at that time. She had never wanted children as she considered them "a nuisance and always cluttering up the house." Her menses started at 13 years of age and were regular and normal until 3 years before admission when she started to miss periods occasionally. Her last period was about 6 weeks before admission. In recent months she has had headaches and dizziness, but no hot flashes. Physical examination at the time of admission revealed a poorly nourished, asthenic female, with a sallow anemic appearance and scattered areas of brownish pigmentation on the face, trunk, abdomen and mucous membranes of the mouth. Roentgenograms of the chest and adrenals revealed no evidence of tuberculosis. The blood count showed a marked hypochromic anemia. The hemoglobin was 8.5 mg. (Sahli). The R.B.C. was 5,200,000 and the W.B.C. 21,600. Differential was normal. Cholesterol was 222 mg. and blood chlorides 408 mg. The patient

was too uncooperative for B.M.R. The patient showed marked agitation and motor restlessness, self-accusation and somatic delusions. She refused to eat and had to be tube fed. Progynon-B Im. was given in doses of 150,000 I.U. weekly in three doses of 50,000 U each. A total of 750,000 I.U. was given. The patient became more agitated and resistive for the first 4 weeks after estrogenic treatment was started. She exposed herself, masturbated, was resistive and had to be tube fed. In view of the low blood chloride, scattered areas of pigmentation and blood pressure of 102/72 it was felt that there might be some adrenal dysfunction which was part of the endocrine picture. Sodium chloride in doses of 4 to 10 gm. daily was given in the tube feedings and intravenously, without any change in the physical findings or mental picture. The intense agitation and tension showed signs of lessening in the 4th week of estrogenic therapy. Vaginal smear, PH of vaginal secretion and Lugol's stain to the vaginal mucosa indicated at this time that the progynon was effective. The patient died following 2 days of vascular collapse with intense diarrhea; a reaction interpreted as primarily adrenal in etiology. Cortin and intravenous saline were given without success.

Pathological Findings (reported by Dr. M. M. Canavan in collaboration with Dr. Shields Warren). The lungs showed a recent bronchopneumonia in the lower lobes. The heart weighed 300 gm. and appeared normal on microscopic examination. The liver weighed 1670 gm. and showed some fatty degeneration. The left kidney weighed 210 gm. and the right 165 gm. There were a few fibrosed glomeruli with casts in the tubules. The ilium and lower jejunum showed no ulceration or evidence of bleeding. There were cells thought to be *Entamoeba histolytica* in the mucosa and sub-mucosa, although there were no ingested red cells or evidence of vacuolization. (The clinical course was not that of amoebiasis.) The pancreas weighed 55 gm. and showed only postmortem changes. The thyroid weighed 17.5 gm. and showed nothing significant. The parathyroids were not unusual. The brain weighed 1250 gm. The posterior cerebral veins were enlarged and filled with postmortem clot. There was no evidence of gross pathology in the brain. The uterus showed blood in the endometrium. (Patient had not menstruated since admission.) The right adrenal weighed 8 gm. and the left 9 gm. The adrenal cortex of both was endematous and gray instead of yellow. Microscopic examination showed a definite decrease in lipoid. The zona glomerulosa was narrow, showed spaces between the acini, and in many places nuclear loss, with dull, cloudy cytoplasm. The zona fasciculata was composed of long columns of cells. Here and there the limiting membrane was all that was seen, with no cells present; a more uneven preservation than the zone above. The vascular sinusoids were unevenly distended. The zona reticulata was very vascular. The cells of the layer were separated and none contained pigment. Medullary cells were arranged in groups and showed some shrinking and fragmentation with slight vacuolization. The sinusoids of the pituitary were fairly well filled in the anterior lobe. There was considerable loss of protoplasm of the cells near the periphery of the anterior lobe. There were many chromophobes. Here also were cells not staining too deeply which showed holes. Droplets of secretion occurred. Higher in the lobe there was a circumscribed area of cells which were large, stained more deeply with hematoxylin and were arranged in an adenomatous arrangement (not adenoma), but had no holes, while on the other side a group of blue cells somewhat smaller, in feebler arrangement showed holes in many. The acidophiles were plentiful in the middle and posterior portion of the anterior lobe. There was some increase of fibrous tissue near the major vessels. The pars intermedia showed several colloid-bearing cysts, with some blue cells of large size outside one of the cysts. The lining cells of the other cysts were

small and numbered up to three cells. The posterior lobe showed an area of invading blue cells with a rich number of pituicytes. In the opinion of Dr. Warren, no symptoms could be attributed to the adenomatous arrangement of chromophobe cells in the anterior lobe of the pituitary. The ovaries were corrugated and on section the edge was crenated. The left weighed 3½ gm. while the right weighed 2½ gm. They were devoid of follicles. There was scarring in the central portion.

Although this patient received 750,000 I.U. of progynon-B Im. over a period of 6 weeks, there was no evidence of pathology in the pituitary which could be attributed to the inhibitory effect of this hormone. The adrenal pathology is more significant in this particular case and raises the question of polyglandular relationships during the involutional period which may become irreversible, show little pathological change, and yet produce profound physiological disorder.

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2. Kirkelia, Q. L., and R. M. Wilder: *Proc. Staff Meet. Mayo Clin.* 11: 121. 1936.
3. Eskin, I. A.: *Bull. de Biol. et Med. Exper. de l'U.R.S.S.* 1: 174. 1936.

CLINICAL AND LABORATORY NOTES

CLINICAL USE OF A NEW ESTROGEN

R. R. Greene and E. M. Dorr*

Successful therapy with the estrogenic substances now in clinical use necessitates intermuscular injections 2 to 4 times weekly in the majority of cases. Oral therapy is of value in some instances, but the cost per effective unit of the present available forms makes their use, except in a very few patients, financially prohibitive.

Estradiol dipropionate, the di-esterified form of the probably true follicular hormone, was synthesized by Meischer and Scholz in 1937.[1] Its enhanced and very prolonged physiological effect over that of estrone, estradiol, estrone benzoate, and estradiol benzoate has been well demonstrated in the experimental animal.[2,3]

Due to the very prolonged physiological effect of this substance, it was thought that it would be particularly valuable in the estrogenic therapy of certain clinical conditions.† It has been given in oil solution by intermuscular injection to 61 patients‡ at the time of writing. Forty-six of these patients were menopausal, 6 had senile vaginitis, and 9 had infantile vaginitis.

In the menopausal series an effort has been made to render the patient symptom free and then determine her maintenance dosage, both as to the amount necessary per injection and also as to the necessary frequency of treatment. As in other reported series of this type, a large individual variation in adequate dosage has been noted. In this small series no definite correlation between the length of time the patient has had symptoms and the adequate dosage has been observed. Thirty-two patients have been observed long enough to formulate some tentative conclusions as to adequate maintenance dosage. Eleven of these patients had previously received varying doses of estrone or estradiol benzoate 2 to 4 times weekly with slight or only moderate relief of symptoms. Of these 32 patients the adequate maintenance dosage has been found to be 5.0 mg. of estradiol dipropionate once a week in 7 patients, 5.0 mg. every 10 days in 3 patients, and 5.0 mg. every 2 weeks, or less frequently in 10 patients. Using the smaller dosage of 1.0 mg. the frequency of injection requisite for relief of symptoms has been once a week in 5 patients and once every 2 weeks, or less frequently in 7 patients.

From the viewpoint of the total dosage required, sufficient data are not yet available to warrant a statement regarding the comparative effectiveness of estradiol

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[1] K. Meischer, and C. Scholz: *Helv. Chim. Acta.* 20: 263. 1937.

[2] K. Meischer, C. Scholz and E. Tschopp: *Biochem. J.* 32: 725. 1938.

[3] K. Meischer, C. Scholz and E. Tschopp: *Biochem. J.* 32: 141. 1938.

† A generous amount of estradiol dipropionate has been made available to us through the courtesy of Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc.

‡ The patients in this series were treated in the Gynecology dispensary service of Dr. H. O. Jones at St. Luke's Hospital.

dipropionate, estradiol benzoate and estrone in cases of menopausal syndrome. From the viewpoint of the requisite frequency of injection, estradiol dipropionate is definitely the most efficacious.

Symptomatic relief and objective cure has been obtained in senile vaginitis with 1.0 mg. once a week, in from 2 to 4 weeks time.

In the treatment of infantile vaginitis we have been of the opinion that intramuscular injection of an estrogen is preferable to estrogenic treatment with vaginal suppositories in that less psychic damage or "genital fixations" occur as a result of treatment. It has been necessary in the past to inject these children at least 2 to 3 times a week.

Of the 9 cases of infantile vaginitis treated with estradiol dipropionate, to date, 5 have been gonorrheal and 4 non-specific (weekly smears consistantly negative for the gonococcus). Definite conclusions as to the amount and frequency of treatment cannot be made because of the small number of patients and the too short period of observation. However, judging by the rapid change to and the persistance of a vaginal smear consisting exclusively of the adult type of squamous epithelial cells, a dosage of 1.0 mg., repeated after 7 days and then at 14 day intervals for a total of 4 or 5 doses is sufficient in the larger children. Correspondingly smaller doses should suffice in the smaller children.

From these clinical findings we feel that we can conclude that estradiol dipropionate has a prolonged physiological effect in the human and is valuable in the treatment of menopausal symptoms, senile vaginitis, and infantile vaginitis.

PHYSIOLOGICAL REACTIONS PRODUCED BY GONADOTROPIC HORMONE FROM THE URINE OF A CASE OF CHORIO-EPITHELIOMA

HARRY L. FEVOLD, VIRGINIA MAYO FISKE AND IRA T. NATHANSON*

EVANS AND SIMPSON[1] have reported that large doses of pregnancy urine hormone (PU) will increase the ovarian weights of immature rats much beyond the limit of 60 or 70 mg. originally given as the maximum stimulation, when administered over a period of 4 or 5 days. The same ovarian response was obtained when they used comparable doses of the gonadotropic substance found in the urine of men with testicular neoplasm. A further comparison of the gonadotropic substances from these two sources showed that neither increased the weight of the testes of pigeons when given in small doses but both had very pronounced effects when given in doses above 5000 R.U. These facts support the general belief that the active substances from these two sources are identical.

We have studied the gonadotropic material from a case of testicular chorio-epithelioma and have found, in agreement with Evans and Simpson,[1] that the ovarian weights increase very gradually with dosage up to 58 mg., when less than 2500 U are given. When 5000 U were administered however, the ovaries weighed 200 mg., while 10,000 U elicited no greater response. The ovaries, qualitatively, showed the typical PU response, characterized by follicles and corpora lutea with the latter predominating (table 1).

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[1] EVANS, H. M., AND M. E. SIMPSON: *Anat. Rec. Supplement*, 17: 61. 1936.

TABLE 1. EFFECT OF INCREASING DOSAGE OF GONADOTROPIC HORMONE FROM THE URINE OF A PATIENT WITH TESTICULAR CHORIO-EPITHELIOMA ON THE OVARIES AND SEMINAL VESICLES OF RATS

Dosage (mg.)	00	.0006	.002	.01	.05	0.2	0.6	1.2	2.4	5.0	10.0
Ovarian weight (mg.)	13	14	20	29	34	40	50	47	58	58	200
Sem. Ves. weight (mg.)	9.5	18	19	26	35	41	56	63		78	

The same material injected into immature male rats was very active. It required approximately one third of a female unit to produce 100% increase in the weight of the seminal vesicles when injected for 5 days into rats 22 days old at the beginning of the experiment.

Five thousand female units injected into each of 3 female pigeons over a period of 5 days, produced ovaries weighing 320, 280, and 210 mg., an increase of from 400 to 700% over those of two controls which weighed 37 and 40 mg. The same amount, injected into 4 male pigeons, resulted in testicular weights of 130, 100, 120, and 240 mg., compared with 26 for the uninjected, an increase of 300 to 800%.

As far as we know, no one has reported the effects of such large doses of PU or its apparent equivalent from the urines of persons with testicular neoplasm, on hypophysectomized rats. The material was therefore tested on rats whose pituitaries were removed at 28 days of age and allowed a postoperative period of 7 days before the beginning of injections. In moderate dosages the chorio-epithelioma hormone produced the typical reaction ordinarily attributed to PU in that no follicular development was produced. The ovaries increased in weight due to the hypertrophy of the interstitial cells, and thecal luteinization had taken place in much the same manner as described by Leonard and Smith[2] for PU. However, when 2000 or more U were given, the ovarian weights increase as much as 1000% and the follicular development was very marked, the ovaries of some rats consisting mainly of cystic follicles while others contained both follicles and corpora lutea (table 2).

In a previous paper we reported that PU could be extracted from urine quantitatively with tri-cresol whereas FSU, the urinary follicle stimulating hormone from menopausal urine, was destroyed by the same method, showing a distinct difference in stability of the two substances.[3] It was therefore possible to determine if the gonadotropic substance excreted in the urine of patients with chorio-epithelioma was similar to PU or FSU with regard to stability and whether or not there was any FSU present, in addition to PU, which might account for the high gonadotropic titre of such urines due to the augmentation effect of the two.

TABLE 2. INFLUENCE OF CHORIO-EPITHELIOMA GONADOTROPIC HORMONE ON THE OVARIES OF HYPOPHYSECTOMIZED FEMALE RATS

Dosage (mg.)	00	.02	.05	0.1	0.2	1.0	2.0	4.0	10.0	20.0
Ovarian wt. (mg.)	6	12	17	15	21	21	24	42[1]	48[1]	68[1]

[1] Follicles and corpora lutea.

The results, however, did not indicate that this was so. Preparations were made using acetone, benzoic acid, and cresol precipitation, and in each case approximately one million female units or about three and a half million male units were obtained per liter of urine. Since no activity was lost with cresol precipitation it does not seem that any FSU could have been present.

[2] LEONARD, S. L., AND P. E. SMITH: *Proc. Soc. Exper. Biol. & Med.* 30: 1248. 1933.
[3] FEVOLD, H. L., AND F. L. HISAW: *Proc. Soc. Exper. Biol. & Med.* 34: 712. 1936.

If, as appears probable, the gonadotropic hormone excreted in the urine of men with testicular chorio-epithelioma and in the urine of pregnant women, are one and the same substance, it seems that the generally accepted idea that this hormone is ineffective in promoting follicular growth in the ovaries of hypophysectomized rats is only true for moderate dosages. This substance can develop follicles if given in sufficient amounts. The only other explanation would depend on the presence of small amounts of FSH which produces its effect only when large doses are given. This however appears unlikely from the experiments reported here. It does not seem probable that the augmentation reaction between FSH and PU would be demonstrated only when such massive doses are given.

Summary. The gonadotropic substance excreted in a case of chorio-epithelioma, when injected for 5 days, increases the weights of the ovaries of immature rats 14-fold, ovaries of pigeons 7-fold, testes of pigeons 8-fold and produced marked follicular and lutein development in the ovaries of immature hypophysectomized rats when given in large doses, (above 2000 u). Further evidence is presented showing this substance to be the same as the gonadotropic hormone found in pregnancy urine.

OVIPOSITOR LENGTHENING OF THE FEMALE BITTERLING PRODUCED BY ADMINISTRATION OF PROGESTERONE

J. J. Duyvené De Wit*

About two years ago Kleiner et al.[1] communicated that the lengthening of the ovipositor of the female bitterling produced by the administration of human urine was due to the male hormone present therein. This statement seemed to be confirmed by positive effects caused by pure androsterone and testosterone. The above mentioned investigators used a method in which the different sterols are put into the water of the aquarium. They dissolve the sterols in propylene glycol and add the solutions to the water of the aquarium.

By the same method I examined the influence of testosterone propionate on the ovipositor lengthing and I also obtained positive results. The curves in figure 1

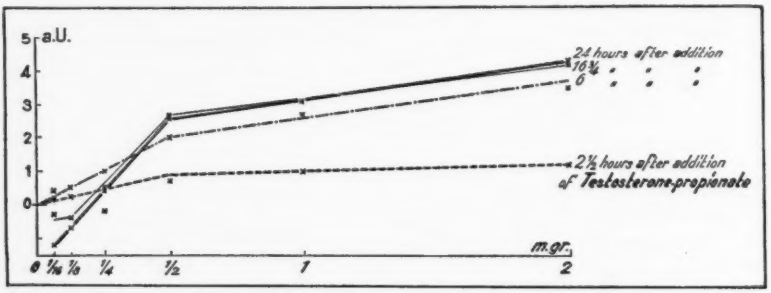

Fig. 1 shows the relation between hormone concentration and ovipositor lengthening, 2½, 6, 16 and 24 hours after addition of testosterone propionate.

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[1] Kleiner, I. S., A. I. Weisman, D. I. Mishkind and C. W. Coates: *New York Zool. Soc.* 21: 4. 1936.

show the ovipositor lengthening 2½, 6, 16¾ and 24 hours after the addition, respectively, of 1/16, 1/8, 1/4, 1/2, 1 and 2 mg. of testosterone propionate to aquaria containing 750 cc. of water (22° C.) The length of the ovipositor was compared with the first ray of the anal fin. If the length of the ovipositor equals the length of the first ray of the spread anal fin the reading is 8 A.U., an ovipositor which reaches only halfway down the first ray is 4 A.U., thus the anal-fin unit, or A.U. is a relative measure, if expressed in mm.

With 1/16 to 1/2 mg. of testosterone propionate the 2½- and 6-hour curves show a direct proportion between ovipositor lengthening and hormone concentration. Then the curves show a more horizontal course.

If the ovipositor lengthening phenomenon is indeed due to male hormones the female hormone progesterone must not show a positive reaction. As I tested 2, 1 and 0.5 mg. of progesterone dissolved in, respectively, 1, 0.5 and 0.25 cc. propylene

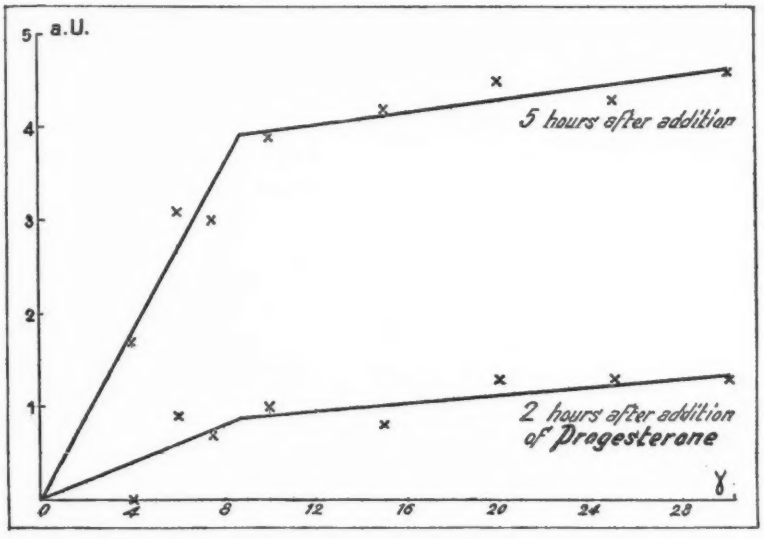

Fig. 2 SHOWS THE RELATION BETWEEN HORMONE CONCENTRATION AND OVIPOSITOR LENGTHENING, 2 AND 4½ HOURS AFTER ADDITION OF PROGESTERONE.

glycol and added to 3 containers with 750 cc. of water, all the fish died within a few minutes. I did not expect such a poisonous effect of progesterone on fish. With lower concentrations I obtained a marked ovipositor reaction as figure 2 shows.

The curves show the ovipositor lengthening respectively 2 and 4.5 hours after the addition of, respectively, 0.04, 0.06, 0.075, 0.07, 0.15, 0.02, 0.025, and 0.03 mg. of progesterone to a number of containers with 750 cc. of water (22° C.), in each of which 2 fish were placed. Between the additional quantities from 0 to 0.009 mg. of progesterone the 2- and 4¼-hour curves show a direct proportion between ovipositor lengthening and hormone concentration.

Comparing the action of testosterone propionate and progesterone it appears that a lengthening corresponding to 1 A.U. is reached within 6 hours by the addition of 0.25 mg. of testosterone propionate or about 0.002 mg. of progesterone to the same

quantities of water. Thus progesterone is about 125 times as active as the tested male hormone. For this reason I do not believe that the ovipositor lengthening is a suitable test for male hormones. It is astonishing that so small an amount as 5γ of progesterone distributed in 750 cc. of water causes a distinct growth of the ovipositor.

Further investigations showed that pregnenes, pregnanes and allo-pregnanes in general cause an ovipositor lengthening. It appeared that pregnenes are more active than allo-pregnanes. In turn allo-pregnanes are more active than pregnanes. The -diones are more active than the -olones. In turn the -olones are more active than the -diols. Desoxycorticosterone-acetate, in a dosage of 2γ per 750 cc. of water is most active of all. Corticosterone is much less active.

Summary. In the female bitterling test (ovipositor lengthening) progesterone appears to be about 125 times as active as testosterone propionate. Pregnenes, pregnanes and allo-pregnanes also give an ovipositor lengthening, desoxycorticosterone-acetate being most active of all.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

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BOOK REVIEWS

PHYSIOLOGIE DER INNEREN SEKRETION. Asher, Leon. Franz Deuticke, Leipzig and Wien, 1936.

The author undertakes to set forth our present-day knowledge of the physiology of the glands of internal secretion as a subject in its own right rather than as an adjunct to clinical pathology. The primary emphasis is laid, therefore, on the hormones themselves, their chemical nature and their quantitative relationships to the biological processes. In an opening chapter is discussed the comparative physiology of internal secretions—interpreting the expression broadly. Interesting topics are the dynamics of internal secretions and reaction patterns. Following this is a section on the adrenal glands in which is evident both the author's first-hand knowledge of the subject and his familiarity with the literature. The next section, that on the thyroid and the parathyroid glands deals searchingly with the part played by their hormones in bodily integration and, especially, in relation to the nervous system. The metabolic aspects receive careful attention. The section on the thymus lays special emphasis on the work of the author and his colleagues on thymocresin. The section on the sex hormones is especially satisfactory and that on insulin is thorough and stimulating. The closing chapter, dealing with the hypophysis, though relatively brief, takes up a wide range of topics.

The Monograph is well printed and readable. The illustrations, many of which are original, are clear and instructive. The book is enriched throughout by the author's first-hand knowledge, gained through many years of research and by his insight into the problems of endocrinology.

IODINE AND THE INCIDENCE OF GOITER. McClendon, J. F. University of Minnesota Press, Minneapolis, 1939.

This monograph of 116 pages deals especially with the world distribution of iodine in soils, water, air, plants and animals; with the world distribution of goiter and cretinism; and with the methods for the microdetermination of iodine.

The chapter on the distribution of iodine is a very complete abstract of the literature, particularly of the past 20 years, including the author's extensive work in this field. Despite the fact that most of the data were obtained by varied and still imperfect chemical methods they, in general, support the thesis of an inverse relation of iodine to endemic goiter, which improved methods of analysis will probably not materially modify. The influence of improved analytical methods on iodine values is vividly illustrated in the rapid decline of the published values for normal human blood iodine during the past 5 years from a previous average of 10 to 20 γ to 5 to 6 γ per 100 cc. at the present time.

The chapter on the distribution of goiter also contains a wealth of references to the recent literature but deals very frugally with the older literature, and yet by contrast with the work on the distribution of iodine the geographical distribution of goiter is an old subject with no important changes (except lessening of its incidence) during the past 100 years. This chapter appears sketchy despite the great number of references cited. A critical evaluation of the data presented and a summary would enhance its usefulness. Fully as detailed and yet a clear picture of the distribution of endemic goiter is given in Hirsch's "Handbuch der historisch-geographischen Pathologie," 1881. No attempt is made to distinguish between endemic and sporadic goiter. Brazil is not mentioned as containing endemic goiter areas and the St. Lawrence River valley is referred to only indirectly, although Osler and Adami emphasized the frequency of goiter in this area nearly half a century ago. The distribution of exophthalmic goiter (which the author sharply separates from 'toxic adenoma'—'Iod-Basedow') is also sketchy. No mention is made that Graves' disease is rare in tropical and subtropical areas (Mills) and that it is more common in urban than in rural populations, or that a high incidence of Graves' disease and endemic goiter co-exist only in highly industrialized, densely populated regions like our own Great Lakes basin and central England.

The author is at his best in the final chapter on the determination of iodine. Because of his many contributions and large experience in this field he frankly admits that "There is no agreement on the adequacy of methods at present available for the quantitative estimation of traces of iodine." This chapter is an excellent summary of the evolution of methods and should be widely consulted by workers in this field.

On the whole the reviewer gets the impression that the greatest value of this work will be as a reference volume for specialists in this field because of its detailed reference to and extensive bibliography of the literature of the present century. A critical summary of the chapters on distribution of iodine and goiter similar to that of the chapter on iodine determinations would greatly extend the usefulness of this monograph.

SEXUAL-HORMONE. Oppenauer, R., and F. Dessau. Tabulae Biologicae Band 15, pp. 121. The Hague, 1938.

One of the series of *Tabulae Biologicae* which present physiological and biological material in tabular form with the absolute minimum of the conventional text. Part I, by Dr. Oppenauer, concerns the occurrence, determination and constitution of the sex hormones. Constants such as melting point and specific rotation that have been determined, are included as well as empirical and structural formulas. Part II, by Dr. Dessau, concerns the biological activity of the sex hormones. The findings of various workers have been classified and organized in such a way that the tables should serve as an excellent survey and index of the great number of papers which have been published in this field.

ADVANCE ABSTRACTS

ALPERT, B., AND E. A. FERGUSON. Local lipoid atrophy and lipoid dystrophy. *To appear in this Journal.*

Out of 430 insulin-treated diabetic patients 32 or 7.4% developed local lipoid atrophy. During the period of dystrophy, if the inoculations are continued, the dystrophy continues and the development of atrophy is delayed. It appears more desirable to use the local site, which has become insensitive to pain, than to use multiple sites for inoculations.

BERGMAN, A. J., AND C. W. TURNER, WITH THE ASSISTANCE OF P. T. CUPPS. A comparison of the guinea pig and chick thyroid in the assay of the thyrotropic hormone. *To appear in this Journal.*

Studies of 10 guinea pigs and 20 male and female chicks were injected with increasing amounts of the initial extract of the AP. In the case of the chicks it was noted that the mean normal thyroid weight of the males was distinctly lower than the mean weight of the females. Further the regression equations calculated for the experimental observations showed that the slopes of the lines were twice as great for the males as for the females indicating greater sensitivity in response to the thyrotropic hormone in the former. Three additional groups of male chicks and guinea pigs were injected at the unit dosage to determine the reproducibility of the assay methods. In both cases the results were considered satisfactory. The male chick, female chick and guinea pig units are defined.

BRACK, C. B., AND O. R. LANGWORTHY. The effect of the estrogenic hormone on vesical muscle. *To appear in this Journal.*

The authors administered the estrogenic hormone to castrated cats whose normal vesical capacities had been previously determined. At the end of one week there was a significant diminution in vesical capacity in 75% of the animals. In only 5% was there an increase in capacity. Following cessation of the hormone, the vesical volumes returned to normal. These findings suggest that in the presence of the estrogenic hormone the vesical muscle tone is increased.

CULMER, C. U., A. J. ATKINSON AND A. C. IVY. Depression of gastric secretion by the APL fraction of pregnancy urine. *To appear in this Journal.*

A study of the effect of the parenteral administration of the APL fraction of human pregnancy urine on the gastric secretory response to a test meal was made in 5 female dogs with Pavlov pouches. After suitable control tests, from 1,000 to 2,000 R.U. of antuitrin-S and 5,000 U. of follutein were given one-half hour before the test meal. In 4 of the 5 dogs, in 7 out of 8 tests, significant to marked decreases in volume and acid output occurred each day during a 5-day period of treatment. In 4 of the 7 instances the secretory response returned to normal one day after cessation of the treatment; in the other 3 the return was slower. 'Estrus' occurred in 4 of 8 instances from 5 to 18 days after the administration of the first dose of APL. Heating the ampoule containing the APL fraction for 90 minutes at 100° C. inactivated the substance which depressed gastric secretion; heating similarly for 10 minutes did not inactivate it. It is concluded that the APL fraction of human pregnancy urine contains a substance which depresses the gastric secretory response of a dog to a meal. The nature of the substance and its mechanism of action is problematic.

ENGLE, E. T., AND P. E. SMITH. Certain actions of testosterone on the endometrium of the monkey and on uterine bleeding. *To appear in this Journal.*

The experiments here reported were performed on adult castrate female rhesus monkeys. The usual uterine bleeding of estrogen privea may be prevented by 5 mg. daily of testosterone propionate. When estrogen was followed by progesterone, the uterine bleeding was not prevented by either 5 mg. or 10 mg. daily doses, but was postponed by 25 mg. of testosterone propionate for 10, 15 and 20 days respectively. The endometrium of animals prepared with estrogen and followed by testosterone propionate is essentially a proliferative type. The endometrium of estrogen-progesterone treated animals, when followed by testosterone propionate, 25 mg. daily for 10 to 20 days, is highly secretory (progravid). There was no endometrial atrophy under any of these conditions.

EVERETT, J. W. Spontaneous persistent estrus in a strain of albino rats. *To appear in this Journal.*

A strain of rats is shown to have developed a strong tendency toward persistent ovarian follicles and accompanying vaginal cornification. This condition appears early in life in a high proportion of the population, becoming increasingly prevalent as the animals become older. In the same population, there is also a pronounced tendency toward delayed and difficult parturition. The continuous supply of estrin in animals which remain in estrus for long periods is at a sufficiently high level to produce changes in the uterus and tubes, simulating those which result from the continuous administration of large amounts of estrogenic substances.

FEVOLD, H. L., AND V. M. FISKE. The inhibition of the follicle stimulating hormone by the pituitary. *To appear in this Journal.*

Estrogen and luteinizing hormone preparations inhibit the action of FSH on the ovaries of immature rats when injected for 8 days previous to FSH treatment. In hypophysectomized immature rats, estrogen does not produce this inhibition whereas LH is as active as in normal rats. Estrogen therefore produces the effect by stimulating the pituitary to secrete the same substance which is administered to the rats in the LH preparations. The inhibiting substance does not produce inhibition by direct action on the ovaries making them refractory to FSH since the ovaries from rats which had not been treated with estrogen for 8 days responded to FSH when implanted into animals which had not been treated with estrogen. On the other hand, ovaries from normal rats implanted into treated rats did not respond, showing that the bodily environment determined the responsiveness of the ovaries. Assay of the blood of rats treated with estrogen for 8 days showed that LH was present in greater concentration than in normal rats or in rats which had received estrogen for only 3 days. Correlated with this fact is the augmentation of the FSH action after 3 days and inhibition of FSH action after 8 days of estrogen treatment. Attempts to separate the anatagonistic principle from the luteinizing hormone were unsuccessful. It seems that the inhibiting agent is either the LH or a factor which is closely associated with and very similar to it.

Folley, S. J. Interactions of estrone and prolactin; with special reference to the effect of estrone on the pigeon crop-gland response. *To appear in this Journal.*

The inhibiting effect of various doses of estrone on the response of the pigeon crop glands to prolactin has been studied. With a given level of prolactin the absolute amount of crop growth prevented by estrone administration rose to a maximum with increasing doses of estrone which was higher when the daily dose of prolactin was 3 mg. than when the daily dose was 1.5 mg. The percentage inhibition due to estrone administration also rose to a maximum with increasing doses of estrone but the maximum was the same for both levels of prolactin, and for the preparation used was equal to about 54%. The power of inhibiting the crop-stimulating activity of prolactin appears to be a characteristic property of estrogenic hormones and suitable conditions for comparing the potency of other substances with that of estrone are defined. Relatively high doses of crop-stimulating extracts of anterior lobe do not inhibit the vaginal response to estrone in the ovariectomized mouse. Estrone and prolactin exert antagonistic effects on the body weight of the pigeon, prolactin tending to increase the body weight while estrone exerts the opposite tendency.

Gaensbauer, F., and J. T. Bradbury. Comparative potency of commercial anterior pituitary-like preparations. *To appear in this Journal.*

Five commercial anterior pituitary-like products were assayed by a common method to determine their comparative potency. Differences in potency were demonstrated on products all of which were labeled as containing 100 r.u. per cc. Two preparations, antuitrin-S and follutein, are approximately twice as potent as antophysin, APL, and pregnyl. Some of the factors determining the value of the rat unit were discussed. The adoption of a standard method of assay for all commercial preparations would result in more uniform potency and would aid in determining optimum clinical doses.

Hoskins, W. H., J. R. Coffman, F. C. Koch and A. T. Kenyon. The effect of testosterone propionate on the urinary excretion of androgens and estrogens in eunuchoidism. *To appear in this Journal.*

The intramuscular injection of 25 mg. of testosterone propionate per day for periods of 9 to 14 days to 4 eunuchoids brought about rises in urinary androgens from basal levels of 2 to 16 i.u. per day to 44 to 115 i.u. per day and of urinary estrogens from the equivalents of 0.3 to 5 γ of estrone per day to that of 5 to 15 γ of estrone per day. This constitutes a restoration to normal values for both groups of substances. Both androgen and estrogen values fell toward pre-treatment levels when injections stopped. The changes in androgen and estrogen excretion paralleled the physiological effects of testosterone propionate in the organism. In 3 patients in whom the cause of the hypogonadism was unknown the extent of recovery of administered testosterone was 65, 49 and 49%, on the assumption that half of the excreted material by weight is androsterone and half dehydroandrosterone. In a patient whose pituitary was destroyed by a suprasellar cyst, the recovery was 20%. The rise in urinary estrogens to normal values following androgen administration suggests that the urinary estrogens of normal male urine may be derived in part from the metabolism of the testis hormone. However, the increase in estrogen excretion repreaents only 0.06% of the androgen injected.

Koven, A. L., and H. H. Beard. Creatine-creatinine metabolism and the hormones. II. Progestin and antuitrin-T. *To appear in this Journal.*

Commercial progestin, crystalline progesterone and antuitrin-T were injected into young rats before and after castration to study their effects upon creatine-creatinine metabolism. The results were as follows: 1. Castration leads to a temporary creatinuria, but does not affect the concentration of tissue creatine in the rat. Likewise the injection of progestin or antuitrin-T does not change the concentration of muscle creatine. 2. These hormones gives a more intense creatinuria in the castrate as compared to the normal animal. 3. Progestin causes an initial increase followed by a decrease in creatinine excretion in normal animals which is accompanied by an increase in creatine excretion. 4. Antuitrin-T causes an increase in the excretion of both creatine and creatinine in the normal animal. 5. A possible explanation is given of the effect of the sex hormones on creatine-creatinine metabolism.

McCullagh, E. P., J. M. Rumsey and W. K. Cuyler. Excretion of urinary androgens following the injection of testosterone propionate. *To appear in this Journal.*

The results of 121 assays in 16 cases of hypogonadism in man and 6 normal men are reported. Following the administration of testosterone propionate in hypogonadal men, it is seen that there is increase in excretion of androgens, which increase is in proportion to the size of the dose. Increases following 5 and 10 mg. doses of testosterone propionate are maintained for not more than 24 hours while following larger doses they are maintained for several days. In normal men, injection of testosterone propionate is followed by a prompt rise in excreted androgens, then for several days there is a fall and subsequently a second rise to normal levels. A possible explanation of this phenomenon is offered.

McGinty, D. A., L. P. Anderson and N. B. McCullough. Effect of local application of progesterone on the rabbit uterus. *To appear in this Journal.*

Crystalline progesterone introduced directly within the lumen of theelin-sensitized immature rabbits produced characteristic progestational proliferation of the endometrium. Single applications of 0.0005 to 0.005 mg. in 0.1 cc. of peanut oil or lanolin in segments of uterus 3 to 4 cm. in length caused reactions in 72 hours equivalent to those obtained with 0.5 mg. progesterone administered in divided doses intramuscularly in the intact rabbit. Application of 0.000125 to 0.00025 mg. produced threshold endometrial reactions.

Mazer, C., and M. Mazer. The treatment of dysfunctional uterine bleeding with testosterone propionate. *To appear in this Journal.*

The experimental and clinical observations which provide the rationale for the use of testosterone propionate in the treatment of dysfunctional uterine bleeding are discussed. The authors' experience with this agent in the treatment of 38 patients with dysfunctional uterine bleeding leads them to the conclusion that it is a valuable therapeutic addition in the treatment of this condition even when employed in relatively small doses. Sixty-eight per cent of the 38 patients were relieved of the abnormal uterine bleeding during a follow-up period of 4 to 19 months. Doses of 30 to 120 mg. given over a period of a month did not disturb the menstrual rhythm of those who prior to the abnormal uterine bleeding menstruated regularly.

Meyer, A. E., and A. Wertz. The calorigenic efficiency of thyroid material in relation to thyroxin and to iodine content. *To appear in this Journal.*

The oxygen consumption of normal and thyroidectomized rats was determined in a closed circuit metabolic apparatus before and after treatment with thyroid material. It appears that neither iodine alone nor thyroxine, as ascertained by chemical assay, give a uniform means of estimating metabolic activity of thyroid material. The chemical linkage within the molecule seems to be an important factor of influence in the physiologic action.

Meyer, A. E., and M. Yost. The stimulating action on metabolism and heart beat of various thyroid preparations determined in the thyroidectomized rat. *To appear in this Journal.*

Treatment of thyroidectomized rats with various thyroid preparations does not act on heart beat and metabolism in an analogous manner. Doses of thyroxine or thyroid globulin that will elevate the metabolism to levels of the normal animal stimulate the heart rate only half way to the corresponding level. With dried whole thyroid the acceleration of the heart rate precedes somewhat the metabolic increment and reaches hyperthyroid-like tachycardia, when the latter arrives at the normal rat value. Hydrolysis split products of the globulin cause far more pronounced heart stimulation, leading to normal and higher beat figures, while the metabolism lags behind. Considerable stimulation of the heart was occasionally observed with these latter preparations, when the metabolism seemed not to be influenced at all. Although it is evident that split products obtained by hydrolysis from the relatively weak cardiostimulating globulin have a strong heart action, it cannot be said whether the same substances are also present in the original gland, and if so, whether one is dealing with a genuine hormone, or with some secondary products perhaps formed by postmortem changes.

Mirsky, I. A., with the technical assistance of S. Swadesh. The influence of the anterior pituitary on protein metabolism. *To appear in this Journal.*

The administration of a crude alkaline extract of the anterior pituitary results in a decrease in protein breakdown of normal dogs. However, when AP treated dogs are eviscerated, an increase in the rate of protein catabolism is observed instead of a decrease. Similar results are obtained when AP extracts are administered to depancreatized dogs. From the data it is suggested that the anterior pituitary exerts a direct effect on the muscles, which is in a direction opposite to that observed in the intact animal. It is postulated that the synthesis of muscle protein consequent to the administration of extracts of the anterior pituitary may be dependent upon the simultaneous stimulation of the pancreas, since insulin is known to inhibit protein breakdown.

Partington, P. F., and I. T. Nathanson. Action of estrin on the blood pressure of the anaesthetized cat. *To appear in this Journal.*

Both aqueous and oil preparations of crystalline estrin were tested for their effect on the blood pressure of cats under Dial (Ciba) anaesthesia. Estrin in aqueous solution injected intravenously gave an immediate prolonged rise in blood pressure of 10 to 50 mm. of Hg. This was absent if the solution had been made approximately isotonic with crystalline NaCl prior to injection. Estrin in oil solution injected intramuscularly caused a slow drop in blood pressure of 30 to 40 mm. of Hg over several hours. This did not occur if the injection site had been previously denervated. Both of these effects on blood pressure

were obtained equally well when the solvents without the estrin were used. The sex and presence or absence of gonads in the experimental animal was not significant for these findings. It was concluded that estrin per se had no effect on the blood pressure of anaesthetized cats.

RUBINSTEIN, H. S., A. R. ABARBANEL AND A. A. KURLAND. The effect of castration on body weight and length of the male albino rat. *To appear in this Journal.*

Thirty-two albino rats of Wistar Institute stock were divided into 2 groups, one of which was castrated at 22 days of age while the other group served as unoperated controls. Weekly weights and final body weights and lengths, taken at 80 days of life were determined for each animal. All data were statistically computed. In addition, theoretical body weights were determined. Initial body weights showed no significant difference between the groups while final body weights and lengths were significantly less by 25.88 gm. and 10.9 mm. respectively in the castrates as compared to the controls. In addition, while final observed and theoretical body weights were in agreement for the control group, the castrates were found to possess final body weights which were significantly greater than the calculated. It was concluded that castration of immature male rats, leads to a suppression of somatic growth as determined by body weight and length. Body length, however, is more affected than body weight, so that, by 80 days of age the animal has become relatively heavy for its stunted length.

SHUTE, E. Persistence of estrogens in the blood after the menopause. *To appear in this Journal.*

Evidence is presented for the persistence of considerable amounts of estrogens in the blood in the majority of women even long after the termination of cyclical menstruation at the menopause.

SPRUNT, D. H., AND S. MCDEARMAN. Studies on the relationship of the sex hormones to infection II. The effect of pseudopregnancy on the spread of India ink in the skin of rabbits. *To appear in this Journal.*

In this report the spread of India ink in the skin of 14 pseudopregnant rabbits is shown to be significantly less than the spread in 32 adult female rabbits. The inhibiting effect of pseudopregnancy is discussed and similar examples of this are noted from the literature.

TALBOT, N. B. Influence of thyroid hormone on serum phosphatase. *To appear in this Journal.*

The serum phosphatase of untreated hypothyroid children tends to be abnormally low (average 3.0 Bodansky units). It is restored to normal levels (average 8.5 Bodansky units) by thyroid therapy.

THOMPSON, W. O., P. K. THOMPSON, S. G. TAYLOR, III AND W. S. HOFFMAN. The treatment of Addison's disease with adrenal cortex extract. *To appear in this Journal.*

Observations with an adrenal cortex extract in the treatment of Addison's disease show that from 10 to 20 cc. daily will often maintain life for long periods without any other form of therapy, but larger amounts are probably necessary to produce optimum conditions. Early in a crisis a patient may be revived by extract alone. When a crisis is well advanced, extract alone is not effective, presumably because of depletion of the sodium reserves of the body. There is no danger at present of giving an overdose of extract. An adequate dose of the extract appears to produce a more satisfactory clinical condition than sodium salts alone. If necessary, because of expense, the extract may sometimes be held in reserve for crises and patients otherwise maintained by sodium salts with or without a low potassium diet. The dose of the extract should be increased during an infection. If no active tuberculosis is present, patients may be maintained in a satisfactory condition for long periods. Even when tuberculosis is present, striking clinical improvement may be produced with adequate doses of extract. There appears to be some relationship between the nutritional state of the patient and the level of the blood pressure. When the basal metabolism is low, raising it to normal with a suitable dose of thyroid is an important part of the treatment. Under such circumstances we have not observed any aggravation of the disease from its use. There appears to be a relationship between the function of the ovary and the adrenal cortex.

THORN, G. W., AND H. EISENBERG. Studies on desoxy-corticosterone (A synthetic adrenal cortical hormone). *To appear in this Journal.*

Bilaterally adrenalectomized dogs, fed a diet low in Na and Cl, may be maintained in excellent condition by a daily subcutaneous injection of a solution of desoxy-corticosterone acetate in oil. The daily requirement of a dog weighing 10 to 12 kg. is approximately 1.5 mg. Withdrawal of desoxy-corticosterone acetate treatment is followed by an increased renal excretion of Na and Cl, weight loss, K and inorganic P retention, hemoconcentration, a rise in the N.P.N. of the blood, a decrease in the serum concentration of Na and Cl and an increase in the serum concentration of K. The animal becomes progressively weaker and his appetite fails. Resumption of desoxy-corticosterone acetate treatment at this time without added NaCl, corrects the altered electrolyte balance and restores the animal to normal health and vigor. As far as can be determined, desoxy-corticosterone acetate treatment results in all of the changes which are known to follow treatment with potent adrenal cortical extracts.

TURNER, C. W., AND P. T. CUPPS. The thyrotropic hormone in the pituitary of the albino rat during growth, pregnancy and lactation. *To appear in this Journal.*

The change in the thyrotropic hormone content of the pituitaries of normal male and female albino rats during various stages of growth, reproduction and lactation is reported. The pituitaries were assayed by groups using day-old White Leghorn chicks. The Bergman-Turner chick units of hormone were determined from the average weight of the thyroids of the injected chicks. The concentration of the thyrotropic factor in the pituitaries of both sexes increased during the period of rapid growth. However, the females contained only about 50% as much hormone as the males during this period. In the estrous female the hormone concentration was low and continued so during the first half of pregnancy. There was evidence of an increase in hormone concentration during the latter part of pregnancy and a further 40% increase during lactation.

WATERMAN, L., I. E. UYLDERT, J. THOMASSEN AND F. OESTRESCHER. Contribution to the examination of the blood of normal and adrenalectomized dogs in relation to cortin treatment. *To appear in this Journal.*

Observations on adrenalectomized dogs, examined several times during months after the operation, have shown that there are no important changes in either the red or in the white blood cell picture during adrenal insufficiency. The changes in clinical behaviour are much more important than the non-significant differences between the data of the blood cell picture of normal and insufficient dogs. An increase in hematocrit value during severe insufficiency could be demonstrated in only 2 dogs.

WEIL, A., AND B. ZONDEK. The histopathology of the pituitary of the white rat injected with follicular hormone. *To appear in this Journal.*

Injections of dimenformon into white rats continued twice weekly over long periods produced the following changes in the pituitary. I. Total maximal dose 120,000 M.U. injected during 9 to 12 weeks. The anterior lobe was enlarged to about one and a half times its normal size and there was a mild swelling of the cells of the three different types. The proportion of eosinophilic cells was 35 to 30% as compared with 38 to 32% in controls injected with olive oil only. II. Total maximal dose 600,000 M.U. injected during 17 to 30 weeks. The anterior lobe was enlarged to about twice its normal size. There was a mild increase in vascularity. In all the three types of cells which were mildly swollen the Golgi apparatus was markedly enlarged and contained a coarse granular debris. The proportion of eosinophilic cells was diminished to 21 to 28%. III. Total maximal dose 780,000 M.U. injected during 32 to 63 weeks. The anterior lobe measured three to four times its normal size and obtained a maximal weight of 100 mg. The interlobular cleft was markedly widened. There was a very marked increase in vascularity which in a number of cases led to interlobular hemorrhages and death. The cells of all the three types were swollen, their Golgi apparatus was maximally dilated and most of the chromophiles were degranulated.

WIMPFHEIMER, S., AND MORRIS FERESTEN. The effect of castration on tubal contractions of the rabbit, as determined by the Rubin test. *To appear in this Journal.*

The character of tubal contractions of 18 living castrated rabbits was compared with that of an equal number of control animals, by uterotubal CO_2 gas insufflation. After castration, the contractions were less frequent and weaker. The administration of estrogenic hormones to 14 castrated rabbits resulted in an increase in the rate and height of tubal contractions. The addition of progesterone given to 6 of the above animals, sensitized with estrogenic substances, resulted in a diminution of the contraction rate in 3 instances and no variation in the remaining 3. After castration, the tubal mucosa of the rabbits became atrophic and the muscle layer thin. Following the injection of estrogenic hormones and progesterone, the tubal mucosa of castrates became hypertrophied and the muscle layer thicker.

ADRENALS

DE WESSELOW, O. L. V. S., AND W. J. GRIFFITHS. The rôle of the adrenal gland and of the raised metabolism in the production of organ hypertrophy in the thyroid-fed rat. *Brit. J. Exper. Path.* 19: 347. 1938.

The following were used to determine the effect of the adrenal medulla and of raised metabolism upon the size of organs in thyroid-fed rats: 19 normal rats, 7 rats receiving 2:4-dinitrophenol, 18 thyroid-fed rats, 13 adrenalectomized and 11 thyroid-fed, adrenalectomized rats. Adrenalectomy did not affect the weight of the heart, kidneys, thyroid and testes. The thyroid-fed rats showed an hypertrophy of the heart, kidneys, adrenals, liver and spleen; this also applied to the adrenalectomized rats later fed thyroid extract. Thus, it was found that the adrenal medulla has no effect on the organs of rats fed thyroid extract. None of the organs was hypertrophied by the raised metabolism resulting from the dinitrophenol. The authors concluded that the action of thyroid on the organs could not be attributed to its capacity for increasing the rate of metabolism.—Margaret Baptist.

HOFFMANN, F. Action of the adrenal cortex hormone (Corticosterone) on female genitalia (Ueber die Wirkung des Nebennierenrindenhormons (Corticosteron) auf das weibliche Genitalie.) *Zentralbl. f. Gynäk.* 62: 2694. 1938.

Synthetic corticosterone in castrated 600 to 800 gm. rabbits caused growth of the uterus and formation of a secretory endometrium, in periods of 4 to 8 days, with doses of 10 mg. or less daily of the desoxy-corticosterone in oil. This preparation failed to give any estrin action judged by the Allen Doisy test in castrated mice, doses up to 3 mg. daily. When rabbit uteri were pretreated with estrin as for progesterone standardization, the desoxy-corticosterone gave results with 1.0 to 1.2 mg. comparable to 0.5 mg. progesterone. The rabbit uterus responds to larger doses of corticosterone without any estrin. Use of adrenal cortex material of this type has no effect on the ovaries of infantile rats, contrasting with the results previously reported by the author (with Anselmino), for extracts of adrenal cortex. They believe there is in adrenal cortex a gonadotropic material different from the life-maintaining hormone.—E. L. S.

HYMAN, A., AND S. F. WILHELM. Differential diagnosis of renal and suprarenal tumors. *J. Urology* 40: 735. 1938.

The authors summarize their work: "1. The differential diagnosis of tumors commonly found at the upper pole of the kidney and in the suprarenal region has been discussed. Eleven illustrative cases are reported: 2. These tumors have been considered, as follows: a) Cysts; b) Inflammatory exudate or abscess; c) Neoplasm of the upper pole of the kidney; d) Neoplasm in the suprarenal region: (1) arising from the suprarenal gland (2) not arising from the suprarenal gland; e) Splenic enlargement. 3. Tumors arising in the suprarenal region, when they attain a large size, dislocate the kidney, but do not usually distort or obliterate the upper calyces. Intrinsic renal tumors, on the other hand, often encroach upon the pelvic and calyceal outline. Blood in the urine also points to intrinsic renal tumor. 4. Intravenous and retrograde pyelography are of great value in demonstrating renal and suprarenal tumors. Minimal pyelographic changes, such as the flattening or absence of a minor calyx, may be the sole sign of a large tumor. 5. Displacement of the kidney, especially on the left side, is significant. 6. Perirenal insufflation is only of limited value. A case of collapse, following its use, is reported. This method should be employed with great caution. 7. The degree of renal mobility is determined by making roentgenograms in the Trendelenburg and reverse Trendelenburg positions. Fixation of the kidney has been found in perinephritis and in infiltrating carcinoma."—J. C. D.

JUDOVITS, N., AND F. VERZÀR. The absorption of different sugars after adrenalectomy (Die Resorption verschiedener Zucker nach Extirpation der Nebennieren). *Biochem. Ztschr.* 292: 182. 1937.

Adrenalectomy diminishes to one half the rate of intestinal absorption of glucose and galactose but not that of sugars which are only slowly absorbed in the intact animal, like mannose and sorbose. The selectively absorbed hexoses are absorbed most rapidly in the upper part of the small intestine, but after adrenalectomy the difference between the upper and lower part of the intestine disappears. The results confirm the previous conclusions that the adrenal cortex is essential for the selective absorption of some sugars.—E. Bozler.

KATO, K., AND H. E. WACHTER. Adrenal sympathicoblastoma in children: with special reference to the biopsy of sternal marrow and of metastatic nodule in the skull. *J. Pediat.* 12: 449. 1938.

The authors count 179 cases of adrenal neuroblastoma in children from 1880 to 1937 and report a case in a male child, 2½ years old, with clinical, hematologic and pathologic data. The authors give a detailed description of the type cell as seen in smears from materials aspirated from both sternal marrow and cervical lymph node. Typical slightly basophilic cells with relatively large hyperchromatic nuclei and fluid-like cytoplasm were identified as metastatic sympathicoblasts or neuroblasts from the primary focus in the adrenal glands. Identical cells were found in preparations from the cut surface of a metastatic nodule in the skull. Metastatic nodules were also found in the liver. The hematology of adrenal neuroblastoma and its differential diagnosis from chloroleukemia and lymphosarcoma are discussed.—M. B. G.

KEENEY, E. L., J. A. PIERCE AND L. N. GAY. Epinephrine in oil. A new, slowly absorbed epinephrine preparation. *Arch Int. Med.* 63: 118. 1939.

Epinephrine in oil, a suspension of powdered epinephrine base in peanut oil, prepared so that 1 cc. of oil contains 2 mg. of epinephrine, is a preparation that is slowly absorbed. Ten patients with chronic asthma who had been taking frequent daily injections or inhalations of epinephrine hydrochloride received relief from asthmatic symptoms for from 8 to 16 hours with 0.65 to 2 cc. doses of epinephrine in oil. One patient received no prolonged effect from adequate doses. Eleven patients were treated during 1 or more acute paroxysms of asthma. Each received from 0.5 to 1.5 cc. of epinephrine in oil and remained free from asthma for 9 to 16 hours, but more generally for 12 hours. For 1 patient with urticaria and another with serum sickness epinephrine in oil provided relief from symptoms for 12 hours; whereas

previously the effect from epinephrine hydrochloride had been fleeting. The hyperglycemic response to epinephrine in oil in 5 cases was maintained for at least 8 or 9 hours, while the response to epinephrine hydrochloride, though more marked, was usually over in 3 hours. The cardiovascularr esponse to epinephrine in oil in 3 cases was maintained for 8 or 9 hours, while the response to epinephrine hydrochloride, as reported in the literature, is generally over in 45 to 90 minutes. The injections of epinephrine in oil are preferably made subcutaneously. There may or may not be slight d[illegible]omfort at the site of a single administration, but consecutive injections are irritating in some cases. N[illegible]merous intramuscular injections can usually be made without following discomfort.—I. B.

Laszt, L. The absorption of amino acids from the intestine following adrenalectomy (Die Resorption der Aminosaüren aus dem Darm nach Nebennierenexstirpation). *Arch. f.d. ges. Physiol.* 240: 636. 1938.

Glycocol is absorbed more rapidly from the gut of the normal rat than are serin, alanin, isoleucin or valin. This differential rate of absorption is lost following adrenalectomy.—A. G.

Laszt, L., and F. Verzàr. The disturbances of carbohydrate metabolism after removal of the adrenal cortex and their relation to Na-metabolism (Die Strärungen des Kohlenhydrat-Stoffwechsels bei Ausfall der Nebennierenrinde und ihr Zusammenhang mit dem Na-Stoffwechsel). *Biochem. Ztschr.* 292: 159. 1937.

Whereas glucose is absorbed from intestinal loops much more rapidly than other sugars like xylose this selective absorption of glucose was abolished in adrenalectomized rats. Three to 8 days after adrenalectomy the animals died with symptoms of severe diarrhea if 2.5 gm. glucose was given per os. Death also occurred following subcutaneous injection of the same amount of glucose. This reaction resembled that resulting from iodoacetic acid poisoning which also produces a loss of the specific resorption of glucose. Adrenalectomized animals could be protected by cortin if administered one to several hours previous to the feeding. Adrenalectomized animals lost much water and Na through the gastro-intestinal tract if glucose was administered. It is believed that this is due to a disturbance of the osmotic equilibrium by the slow absorption of glucose. Because active processes probably determine the entrance of glucose not only into the intestinal wall but into cells in general. This explanation can also be applied to the loss of Na from the tissues after adrenalectomy.—E. Bozler.

MacKay, E. M., and H. O. Carne. Influence of adrenalectomy and choline on the fat content of regenerating liver during fasting. *Proc. Soc. Exper. Biol. & Med.* 38: 131. 1938.

Fat is deposited rapidly in regenerating liver of partially hepatectomized fasting rats, the maximum being reached in 24 hours. The rate and amount of fat deposited in regenerating liver was found to be greater in females than in males. Adrenalectomy and/or glucose administration reduces the deposition of fats. Choline is without influence upon the rate or degree of fat deposited in regenerating livers of partially hepatectomized rats.—E. T. Gomez.

Marenzi, A. D., and O. Fustinoni. Potassium in blood and tissues of adrenalectomized toads (El potasio de la sangre y de los tejidos de los sapos suprararrenoprivos). *Rev. Soc. argent. de Biol.* 14: 118. 1938.

The adrenals were destroyed by diathermo-coagulation; in controls the kidney was cauterized without injury to the adrenals. The K was determined by Marenzi and Gerschmen's technic after nitroperchloric calcination. In blood serum 48 to 72 hr. after adrenalectomy a 24.4% decrease in Na and a 33.9% increase in K was observed. The concentration nerve (21.9%), it was not significantly altered in muscle (gastrocnemius).—J. T. L.

Rogoff, J. M., E. N. Nixon, G. N. Stewart and E. Marcus. Epinephrine secretion in hypophysectomized dogs. *Proc. Soc. Exper. Biol. & Med.* 37: 715. 1938.

The rate of epinephrine secretion in the adrenals of 21 hypophysectomized dogs, including 2 acute and 19 survival experiments, was determined by the method of Stewart and Rogoff. In survival experiments, epinephrine determination was carried on at various intervals beginning from 2 to 165 days after hypophysectomy. The rate of liberation of epinephrine was not affected in the acute experiments. In survival experiments the hypophysectomized dogs showed marked atrophy of the adrenals; the rate of blood flow from the adrenal was decidedly lower and the concentration of epinephrine was correspondingly greater than in normal dogs. The increased epinephrine in the adrenal vein blood was not taken as an indication of increased secretion but merely a concentration due to the diminution of blood flow from the glands. It was concluded that the epinephrine secretion is not significantly altered by hypophysectomy.—E. T. Gomez.

Sato, H., T. Muto and M. Hatano. How atropine interferes with the epinephrine secretion causable by morphine or insulin. *Tohoku J. Exper. Med.* 34: 393. 1938.

Dogs were used to determine the effect of atropine upon the acceleration caused by morphine and

insulin in the secretion of epinephrine. Atropine, which was given in doses of 5 mg. per kg. of body weight, was followed in some of the dogs by 4 to 10 mg. of morphine per kg. of body weight, in others by 7 U of insulin per kg. of body weight. Epinephrine was estimated by the rabbit intestine segment method. Its rate of output was not inhibited by the atropine; in fact, it was accelerated nearly as much as by morphine or by insulin alone.—Margaret Baptist.

ENDOCRINE GENERAL

Boon, A. A. Comparative anatomy and physio-pathology of the autonomic hypothalamic centres. *Suppl. 18. Acta psychiat. et neurol.* Pp. 129, 1938.

Boon offers an extensive review of the literature and reaches several conclusions that are significant for endocrinology. The hypothalamus is a highly important center for the regulation of vegetative and hormonal mechanisms but sometimes considerable lesions are found in the hypothalamus, without noteworthy vegetative disturbances, e.g., in cases of slowly growing tumors. In some vegetative functions the lower (bulbar and spinal) autonomic centers, though being connected with it in high vertebrates by descending hypothalamic tracts, apparently may sufficiently cooperate in balancing these functions, independently from it. This also appears from the fact that in lower vertebrates, fishes especially, the descending hypothalamic autonomic tracts are still poorly developed. On the other hand it is evident that several vegetative functions (e.g., hypophyseal and thermoregulatory)are strongly influenced by the hypothalamus and that the cortical (and striatal) influences on lower autonomic centers have synapses in this region, thus explaining also the increase of these centers, especially in the post-infundibular region, in mammals.—R. G. H.

Elman, R. Urinary output of nitrogen as influenced by intravenous injection of a mixture of amino acids. *Proc. Soc. Exper. Biol. & Med.* 37: 610. 1938.

Seven healthy adult dogs were each given hourly by intravenous injection, until a total daily dose was given, a mixture of 10% amino acids containing 10% glucose beginning from 3 to 7 days following a preliminary starvation period. The amino acids used were obtained by the acid hydrolosis of casein. All experiments were conducted in consecutive 3-day periods. Dogs receiving complete amino acid mixture (basic amino acid with 2% tryptophane and added cystein) either with or without excess nitrogen immediately establishes the N-balance and persists through the period of injections of the mixture. Those which received the basic amino acid mixture alone did not establish a N-balance in spite of the fact that the amount of nitrogen was the same in both experiments. Further, dogs on basic mixture treatment showed increased urinary N output which persisted 24 hours following cessation of injections. This condition does not occur in dogs receiving complete amino acid mixtures. These observations were interpreted as indicating that the mixture of amino acids with 2% tryptophane and added cystein was efficiently utilized by the body.—E. T. Gomez.

Greengard, H., and A. C. Ivy. The isolation of secretin. *Am. J. Physiol.* 124: 427. 1938.

Secretin has been isolated in the form of a crystalline picrolonate. The picrolonate (C_4H_3ON) melts sharply with decomposition at 234–235°C. and recrystallization may be repeated without altering the activity. Although the molecular weights of the free secretin or its derivative were not obtained, the authors believe their active compound to be a simpler molecule than that isolated by Hammarsten and his coworkers. The authors found that a large part (80%) of their picrolonate is picrolonic acid, which was checked by the fact that the free secretin base by direct assay had a dog unitage of approximately one-fifth the dosage of the picrolonate. Deproteinization was accomplished by acetone-aniline treatment before formation of the derivative. A secretin hydrochloride gave negative color reactions to the ninhydrin, Hopkins-Cole and Millon tests and a doubtful response to the biuret test. Limited studies indicated the free secretin to be very weakly basic, to probably have no free amino or carboxyl groups, and to have no reactive hydrogen atoms. It was not oxidized by aldehyde reagents but was completely inactivated by permanganate. There was no reaction with acetyl chloride or with methyl magnesium iodide in ether. One hundred pieces of the first 6 feet of hogs' intestines yielded 0.5 gm. of the picrolonate from which 0.08 gm. of free secretin was obtained by acid hydrolysis. 0.014 mg. of free secretin constitutes one Ivy-dog unit.—H. W. S.

György, P. Nicotinic acid and prevention of nutritional panmyelopthisis in rats. *Proc. Soc. Exper. Biol. & Med.* 38: 733. 1938.

Nicotinic acid added to the vitamin B deficient diet of rats resulted in complete suppression of panmyelopthisis, a common complication occurring in rats under this dietary regimen without nicotinic acid. The experiment seemed to show a close relationship between nicotinic acid and the prevention of panmyelopthisis and all of its manifestation.—E. T. Gomez.

HURXTHAL, L. M. Hemochromatosis as a cause of renal and suprarenal failure: a case report. *Lahey Clin. Bull.* 1: 9. 1938.

The case is reported of a man who, for more than 2 years, had experienced serious ill-health as evidenced by weakness and fatigue, poor appetite, over-consumption of alcohol, cramps, loss of weight and vomiting. He showed a marked anemia and moderate leucopenia with a reticulocyte count of 3%. The non-protein nitrogen of the blood was 98 mg. % and the bilirubin 0.1 mg. %. A bone marrow biopsy suggested myelogenous leukemia but the picture was not characteristic. He improved for a time on liver extract and Fowler's solution with iron but suffered a relapse. A skin biopsy then showed hemochromatosis. The main features of the disorder were finally ascribed to hemosiderin deposits in the kidneys and adrenal glands. Specifically supporting the diagnosis were skin pigmentation, low blood pressure, early gastro-intestinal symptoms and favorable reaction to injections of glucose and saline solution.—R. G. H.

KRIMBERG, R. On the rôle of muscle tissue as a source of hormones stimulating secretion (Untersuchungen über die Rolle des Muskelgewebes als Quelle sekretionssteigernder Hormone). *Biochem. Ztschr.* 297: 249. 1938.

Extracts from beef muscles or Liebig's extract injected intravenously into dogs produced secretion of pancreatic juice and of bile. The action had a short latent period and lasted only for a brief period like that produced by secretin. The author discusses his opinion that secretin consists of several substances which act non-specifically on several digestive glands and that skeletal muscles are important for the production of these substances.—Emil Bozler.

NOMLAND, R., E. A. SKOLNIK AND L. L. MCLELLAN. Jaundice from bismuth compounds used in the therapy of syphilis. *J. A. M. A.* 111: 19. 1938.

In an outpatient clinic over a period of five years, jaundice attributed to antisyphilitic treatment developed in 75 cases. Extensive study of the cases was not possible, but the criteria for diagnosis were lack of evidence of syphilis of the liver and of other diseases of the liver or biliary system, development of the jaundice within a reasonable time after the last treatment and complete clinical recovery. For bismuth compounds the time chosen was 6 weeks and for arsphenamine 12 weeks. The criteria for the diagnosis of bismuth jaundice were lack of previous treatment with arsphenamine, at least within the preceding 3 mo.; development of jaundice within 6 weeks after the last treatment with a bismuth compound; absence of other causes of jaundice, and complete clinical recovery. In all cases the bismuth preparation was bismuth subsalicylate given in a dose of 1.5 or 2 cc. of the 10% suspension in oil at weekly intervals. Age, sex, race and season of the year did not seem to have any bearing on the incidence of the jaundice. Of the 22 patients who had had neoarsphenamine but whose jaundice was presumed to be caused by bismuth, 7 had had their last neoarsphenamine from 12 to 16 weeks before, 3 from 16 to 23 weeks before, 11 from 24 to 30 weeks before and one over 30 weeks before. Because of the time which had elapsed since the last injection of neoarsphenamine, the authors felt that neoarsphenamine had nothing to do with the jaundice. Jaundice developed in 4 patients in the first week after the last treatment, in 5 within 1 to 2 weeks, in 3 within 2 to 3 weeks, in 4 within 3 to 4 weeks and in 5 within 4 to 6 weeks. In all except 3 the bismuth preparation used was 10% suspension of bismuth subsalicylate in oil given in a dose at 1.5 cc. Three patients received considerable amounts of bismuth potassium tartrate or bismuth sodium tartrate. The clinical picture in bismuth jaundice is in no way distinguishable from that of other forms of toxic jaundice, such as jaundice from neoarsphenamine, mapharsen, sodium cacodylate or from other drugs. The severity of jaundice from bismuth was variable, but most of the attacks were mild or moderately severe. Of the 10 persons with jaundice who had had only a bismuth compound, 2 had mild, 5 moderate and 3 severe jaundice. Of the 22 who had been given arsphenamine 12 or more weeks and a bismuth compounds 6 weeks or less before jaundice developed, nine had mild, ten moderately severe and three severe jaundice. The incidence of bismuth jaundice over the period of 5 yr. has been one attack for 2,242 injections of bismuth compounds. The incidence of jaundice from neoarsphenamine during the same period has been one attack for each 951 injections.—Abstract Service *J. A. M. A.*

TALBOT, F. B. Basal metabolism of undernourished girls. *Am. J. Dis. Child.* 56: 61. 1938.

During acute undernutrition there are a rapid loss of weight and a corresponding lowering of the heat production. During severe chronic undernutrition there is a lowered production of heat. The greater the degree of malnutrition the less the heat production of the body. The author states that the teaching that malnutrition is accompanied by increased heat production resulted from the error of using standards for metabolism which divide the total amount of heat produced by body weight, or by body surface when weight is used in the formula. Results obtained with such standards are only relative and do not give evidence of the true physiologic status. He further states that the heat production of girls who are between 10 and 20% below their normal weight averages 7.2% less than the total calories for the ex-

pected weight as shown by the height standard. With more severe malnutrition the heat production averages 10.4% less than the total calories for the expected weight and may be as low as −27% below that value. Even though the absolute heat production is diminished, it may appear to be increased if there is a greater proportional loss in inert tissues than in heat production. When standards are based on body weight, as is the case with body surface standards and the 'multiple prediction formula,' the metabolism will appear to be increased or to be diminished according to the composition of the weight lost. In order to obtain a knowledge of the true state of affairs in malnutrition, the absolute total heat production should be compared with standards expressed in the terms of expected average weight for the height. This can be done simply by using the height standard.—M. B. G.

WINKLER, H., AND A. BINDER. Does hormone production depend on ascorbic acid in tissues? (Welche Abhängigkeiten bestehen zwischen Asorbinsäurebeladung und Hormonproduktion?) *Zentralbl. f. Gynäk.* 62: 2738. 1938.

Various endocrine glands of calves were stained for ascorbic acid and the distribution of the granules described. There is no evidence of relations between these dots and secretion.—E. L. S.

GONADS

AYKROYD, O. E., AND S. ZUCKERMAN. Factors in sexual skin edema. *J. Physiol.* 94: 13. 1938.

Observations on the sexual skin changes were made upon 4 female Macaca mullatta monkeys (2 of which had been spayed) and 8 males. All except one female and one immature control male were injected with estrone (usually 100 mg.) daily for varying periods of from 14 to 368 days. India ink, Chicago blue, gelatin-carmine and methylene blue injections into the descending aorta of previously perfused animals showed in both active and inactive sexual skin that this region was clearly demarcated before other regions of the skin gave evidence of the injections. The conclusion is reached that the vessels are more numerous, larger and probably more permeable (methylene blue). Intercellular spaces give a negative mucoid reaction, but the interspace of the collagen fibers are fairly positive. Mast cells seem depleted of their granules in the active phase and it is suggested that they secrete a muco-protein which holds intercellular water. Local injections of trypan red into the active sexual skin showed an even spread of the dye indicating that the interstitial substance was not a gel which prevented the passage of dye. Increased permeability of the region is indicated by the abnormally high protein content of the interstitial fluid.—A. E. Severinghaus

BASTENIE, P., AND S. ZYBERSZAC. Demonstration of the hormonal stimulation by means of colchicine. Demonstration of the stimulating action of testosterone propionate on the seminal vesicle in the rat (Mise en evidence de stimulations hormonales par la colchicine. Detection de l'action stimulatrice du propionate de testosterone sur les vesicules seminales du rat). *Compt. rend. Soc. de biol.* 126: 891. 1937.

Immature rats were injected with 3 mg. of hormone on 3 successive days and killed on the fourth. One group of these received 0.025 gm. of colchicine per 20 gm. of body weight 9 hours before they were killed. A control group was given colchicine without the hormone. In the doubly treated animals there were 22 to 37 mitoses in a field compared with 0 to 3 mitoses in those treated with hormone alone. Colchicine by itself had no effect on the rate of mitosis.—J. C. D.

BAUER, W. The treatment of puerperal hemorrhage with follicular hormone (Die Behandlung von Wochenbettsblutungen mit Follikelhormon). *Zentralbl. f. Gynäk.* 61: 2693. 1937.

Bleeding caused by inflammation and delayed healing of the uterine surface was favorably influenced by medication with doses of 10,000 U estrin. The treatment did not permit any diagnostic conclusions as to the source of the bleeding.—A. E. M.

BIGLER, J. A., L. M. HARDY AND H. V. SCOTT. Cryptorchidism treated with gonadotropic preparations, III. Surgical repair of cryptorchidism with and without gonadotropic therapy. *Am. J. Dis. Child.* 56: 989. 1938.

The authors report observations on 43 patients operated on for cryptorchidism, 7 patients who were given gonadotropic hormone subsequently and on 22 who were operated upon after having had treatment. They conclude that slightly better results were obtained when a gonadotropic substance was given before operation (64%) than when operation alone was performed (60%). Post-operative treatment with gonadotropic principle had no beneficial effect on the testes in preventing or in correcting atrophy. With all forms of treatment, the best results seemed to occur between the ages of 7 and 10 years. The gonadotropic principle should be given a fair trial in the treatment of boys over 7 years of age. Operation should be considered if complete recovery does not occur after adequate therapy.—M. B. G.

Bischoff, F. Chemical studies on the gonadotropic hormone of pregnant mare serum. *J. Biol. Chem.* 125: 697. 1938.

The gonadotropic fraction obtained by fractional precipitation from pregnant mare serum by alcohol was treated with various chemical reagents and assayed by the effect on 3-week-old male and female rats. In mildly alkaline but not in acid solutions the material was 90% inactivated by acetylation, β-naphthoquinone sulphonate, diazobenzene sulphonate and by iodine. It was relatively stable to excess of nitrous acid, dimethyl sulphate and formaldehyde. The material reacted similarly to prolan under various test procedures and could not be separated into follicle-stimulating and interstitial cell-stimulating components by precipitation with ammonium sulphate.—J. M. L.

Blaisdell, I. H. Use of estrogenic substances in atrophic rhinitis. *Laryngoscope* 48: 699. 1938.

A series of 60 cases is presented, divided into two groups; group 1 consisted of patients with an onset (true atrophic rhinitis) before 20 years of age and group 2 was made up of patients with onset (secondary atrophic rhinitis) of symptoms after 20 years of age. There was clinical improvement as shown by diminution of crusts and odor in 86.4% of the patients in group 1 and improvement of all patients in group 2. Because of its wide variety of effects on the body, aside from its function as a sex hormone, estrogen, properly used, is one of the greatest weapons for combating diseases and physiologic conditions which heretofore have been obscure. Besides acting as a hormone, estrogen acts also as a drug and its action is farther reaching than insulin, more powerful than epinephrine and more spectacular than ergot. The relationship between menstrual abnormalities and atrophic rhinitis should be undertaken by a gynecologist. The exact mechanism by which estrogen acts on the nose is not known and the extent of changes in the mucosa, as well as the permanence of the improvement clinically, can be determined only after years of carefully studying these cases.—From abstract in *J. A. M. A.*

Bottomly, A. C., and S. J. Folley. The effect of high doses of androgenic substances on the weights of the testes, accessory reproductive organs and endocrine glands of young male guinea pigs. *J. Physiol.* 94: 26. 1938.

The paper describes the effects of administration of various crystalline androgens on the weight of the testes, accessory reproductive organs and endocrine glands of immature male guinea pigs. For most treatments 5 animals were used. The hormones were dissolved in sesame oil. Testosterone propionate caused growth of testes for 1 week, then marked regression to 3 weeks, at which time testes weights again showed sharp increase. The author' statement, therefore, that a marked regression of testes weight occurs, covers only a restricted portion of the experimental data. Simultaneous injections of horse AP hormones cancel the effect of testosterone propionate. In daily doses of 2 mg. for 30 days, cis-androstandiol, Δ_5-tr-androstendiol, Δ_4-androstendione, cis-androsterone, dihydrotestosterone, 17-methyltestosterone, and testosterone also caused atrophy of the testes. Testosterone propionate and cis-androstandiol are the most active stimulators of seminal vesicle weight increase, while the former also was most effective in causing growth of the prostate. None of the androgens caused changes in the weights of the hypophysis, thyroids or adrenals and only 17-methyltestosterone seemed to affect body growth, the percentage increase in body weight being significantly greater than that of the controls. In the interpretation of their results, the authors have followed without questioning the frequently suggested idea that the androgens as well as the estrogens inhibit the active secretion of the hypophysis.—A. E. Severinghaus

Brewer, J. I. Rhythmic changes in the skin capillaries and their relation to menstruation. *Am. J. Obst. & Gynec.* 36: 597. 1938.

The Cutter-Johnson negative pressure instrument was used to test capillary fragility on the forearm. In women with regular and presumably ovulatory menstrual flowing there is a striking increase in fragility, with petechial hemorrhages on testing, occurring during the few days prior to and on the first day of menstruation. These changes are attributed to vascular spasm. In patients with functional and anovulatory bleeding the capillary changes were dissociated from the menstrual rhythm. The author concludes that the vascular phenomena of menstruation are not limited to the uterine vessels.—E. L. S.

Butz, L. W., and S. R. Hall. Some characteristics of the androgenic fractions from bull urine. *J. Biol. Chem.* 126: 265. 1938.

From 1 liter of bull urine 6 mg. of a ketonic cholestenone fraction was obtained which contained only 0.01% of androgens as active as androsterone. The activity of the androgens in bull urine was thought to be due to α, β unsaturated ketones which are very unstable toward alkalis. The small activity of the cholestenone fraction was caused by contamination with α, β-enones. Milder methods of hydrolysis will be needed to isolate androgens with olefin linkages adjacent to functional groups.—J. M. L.

CARMICHAEL, H. T., AND A. T. KENYON. Eunuchoidism. A psychiatric study of six cases. *Arch. Neurol. & Psychiat.* 40: 717. 1938.

Six men with eunuchoidism were studied as to physical, endocrine and psychiatric characteristics. No typical personality pattern could be recognized. The group showed an average urinary excretion of androgenic and estrogenic substances per day amounting to one-third that of normal persons. During treatment for long periods with an extract of the urine of pregnant women there were observed slight changes in 2 patients and remarkable advances in sexual development, similar to the changes in normal boys at puberty, in another.—R. G. H.

CUNÉO, B., AND J. JOMAIN. Treatment of prostatic hypertrophy with testicular extract (Traitement de l'hypertrophie prostatique par l'extrait testiculaire). *Presse méd.* 46: 913. 1938.

A glycerol extract was prepared containing, in the 5 cc. dose, the equivalent of 0.6 gm. fresh gland and a potency of 3 I.U. or 0.3 mg. androsterone. The treatment consisted of 2 daily doses for 10 to 20 days, one dose for 15 days and an injection every 2nd day for some time to follow. Disregarding the small dose, the results were equal to those obtained with the synthetic hormone. Functional difficulties (pollakiuria) subsided promptly. The success was obtained not only in the initial stages but also in cases of definite prostatic enlargement.—A. E. M.

DEULOFEU, V. Excretion of injected estrone in the horse (Excreción de estrona exógena por el caballo). *Rev. Soc. argent. Biol.* 14: 405. 1938.

The estrone content of the urine of horses was determined by its estrogenic activity in castrated rats and by isolation in the form of semicarbazone; then 100 to 200 mg. were injected subcutaneously and, the urine again examined. In non-castrated horses 60 to 69% of the injected hormone was recovered calculated by the estrogenic activity, and 40 to 69% as semicarbazone. In geldings, which did not eliminate estrone in the urine, only up to 9% of the injected estrone was recovered in the urine.—J. T. L.

DOMANSKI, T. J. Apparatus for the extraction of estrin from urine. *J. Lab. and Clin. Med.* 23: 412. 1938.

An apparatus is described in which chloroform is used as the sterol solvent after acid hydrolysis. Six hours is needed for extraction.—F. A.

EFFKEMANN, G. Hormonal therapy in deficient lactation in the woman (Hormonale Therapie der ungenuegenden Laktation bei der Frau). *Zentralbl. f. Gynäk.* 61: 2686. 1937.

Pregnant women with underdeveloped breasts, giving a prognosis of poor lactation, were treated during the 9th month with 50,000 U follicular hormone every 3rd day for 30 days, followed by 10,000 U estrin with 5 mg. progesterone every 3rd day. The total treatment amounted to 60 mg. estradiol benzoate and 51 mg. progesterone during 2 months. The lactation was uniformly increased as compared with controls. Reduction of dosage did not lead to satisfactory results.—A. E. M.

EMGE, L. A., AND K. M. MURPHY. Effect of rapidly repeated pregnancies on transplantable mammary rat adenofibroma. *Proc. Soc. Exper. Biol. & Med.* 37: 620. 1938.

Fast-growing rat mammary adenofibroma (5-B1) were transplanted into adult male and female white rats. Some of the recipient rats (15 rats) were bred from 2 to 4 times in succession. All tumors were removed after 96 days of growth. Cytological studies of tumors recovered from all female rats showed hyperplastic changes well within the limit of mammary response to pregnancy. Those obtained from the males, however, showed marked diminution of adenomatous components. Further, it was shown that rapidly repeated pregnancy or retention of secretion product of the gland does not induce changes toward malignancy of these tumors.—E. T. Gomez

PANCREAS

COLLIP, J. B. Results of recent studies on anterior pituitary hormone. *Edinburgh M. J.* 45: 782. 1938.

This gives a general view of our present knowledge. The metabolic stimulator which acts without the mediation of the thyroid can now be separated from all the other hormones except that which expands the melanophores. Like this latter, it is found in greater quantities, per unit of gland, in the intermedia than in the other parts. A method of extraction is given. Sheep injected for a considerable period with gonadotropic hormone develop an antihormonal substance. Prior to this, however, their blood contains for a short time a substance which enhances the action of gonadotropic hormone. In rats, injected with gonadotropic hormone, the changes in the gonads and hypophysis indicate that the antihormone reaction following long continued treatment acts not only against the injected substance but also against the animals' own gonadotropic hormone.—J. C. D.

COPE, C. L. The young chick as test for the thyrotropic hormone. *J. Physiol.* 94: 358. 1938.

This report offers considerable data in confirmation of Smelser's suggested use of the chick in quantitative work on the thyrotropic hormone of the pituitary. The consistency of weight of normal chick thyroids was shown by 35 birds ranging from 10 days to 3 weeks in age. The uniformity of the histological picture throughout the gland makes changes in response to extract easy to detect, and the thyroid weight-increase in response to thyrotropic hormone is almost a linear graph when plotted against daily dose in a series of 20 chicks. An experiment on 38 birds shows that they may also be used for work on antithyrotropic sera, the mean increase in thyroid weight after thyrotropic hormone being inhibited by antithyrotropic rabbit serum.—R. L. Z.

DODDS, E. C., S. H. LIU AND R. L. NOBLE. Water balance and blood changes following posterior pituitary extract administration. *J. Physiol.* 94: 124. 1938.

Dodds and Noble have previously reported that the administration of post-pituitary extract would produce anemia in rabbits. In this paper the effect of similar treatment on anemia, water balance, and blood volume were studied on 65 rabbits. Animals were maintained on a dry diet (oats, bran, hay) with water ad lib., or on a wet diet of carrots and swedes or swedes alone. Slight or moderate anemia occurred in almost all of the animals, but 11.5% of them developed a severe anemia. Severe anemias occurred without respect to strain or breed of rabbits, sex, or wet or dry diet. In those animals resistant to severe anemia, intravenous injections were no more effective than the subcutaneous injections, nor did alteration in the spacing and size of doses produce the symptoms. No increase in water balance or blood volume could be observed even in cases of typical anemia. The authors therefore conclude that the suggestion of Gilman and Goodman that the anemia results from water retention which causes blood dilution cannot be supported by the present experiments.—A. E. Severinghaus.

FRANK, S. Studies on the thyroid gland. VII. Histophysiology and endocrine interrelationship of the anterior pituitary. *Acta. path. et microbiol. Scandinav.* 14: 339. 1937.

Injections of alkaline extract of anterior pituitary cause changes in the cytology of the anterior lobe in the guinea pig characterized by increase of the chromophobes, of the hypoacidophil, small acidophil, degranulated acidophil and pyknotic acidophil elements with concomitant reduction of the typical acidophils and of the typical basophils. Colloid follicles are formed as are usually not found in animals of the age as used. Basophil colloid is increased in the posterior lobe. Gonadotropic and adrenotropic extracts cause similar changes with accentuation on basophils. The changes produced by gonadotropic extracts appear also in castrates. Thyroid and adrenal cortex hormones produce in the anterior pituitary degranulation of the acidophil elements, a change not observed after adrenaline. Estrin, testicular hormone and insulin cause degranulation of the basophils. The theory is developed that acidophil and basophil cells represent different secretory or functional stages of the same cell: the anterior pituitary cell.— A. E. M.

MCSHAN, W. H., AND R. K. MEYER. The effect of trypsin and ptyalin preparations on the gonadotropic activity of pituitary extracts. *J. Biol. Chem.* 126: 361. 1938.

The effect of two commercial trypsin preparations, crystalline trypsin and ptyalin preparations on the gonadotropic activity of two unfractionated extracts and a follicle-stimulating extract prepared from sheep pituitary was determined by digesting at 37° C. for 3.5 hours, and assaying on normal 21-day-old female rats. The luteinizing activity of the extracts was largely, if not entirely, destroyed by trypsin, while it was relatively resistant to ptyalin. The follicle-stimulating activity was resistant to trypsin, while it was destroyed by ptyalin.—J. M. L.

HYPOPHYSIS

AUBURTIN, E., A. LACOSTE AND R. SARIC. The action of repeated insulin injections on structure and function of the islets of Langerhans (Action des injections répétées d'insuline sur l'état structurel et fonctionnel du tissue Langerhansien). *Ann. méd.* 43: 253. 1938.

Experiments on dogs showed that repeated insulin injections increase the insular tissue in the pancreas, especially in the head; the effect is most marked during the first months. The treatment results in functional irregularities of the pancreatic endocrine apparatus. The insulin is stored in liver and muscles In the diabetic patient, insulin treatment tends to slow the development of the disease and leads to improvement. This can be observed even in the infantile patient.—A. E. M.

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